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BULLETIN OF THE TORREY BOTANICAL CLUB
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HETEROKARYOSIS, MATING-TYPE FACTORS, AND SEXUAL REPRODUCTION IN NEUROSPORA

EVA RICHARDSON SANSOME

INTRODUCTION

Dodge was the first to describe the experimental synthesis of heterokaryons in *Neurospora* (1942). He used "unisexual" lines of the homothallic species *N. tetrasperma* and found that vegetative heterokaryons, more vigorous than their constituent homokaryons, could be produced, whether the strains were of the same or different mating-types. In *Neurospora crassa* this is not the case. In this species, vigorous heterokaryons can readily be made from two mutants of the same mating-type (Beadle & Coonradt 1944, Sansome in press), but when two such mutants belonging to different mating-types are put together, no appreciable increase in vigor occurs. Beadle and Coonradt suggested that the lack of vegetative vigor in combinations of different mating-types may be due to the "tendency of the hyphae containing nuclei of the two sexes to form fruiting bodies (perithecia) rather than grow in the manner of strictly vegetative hyphae." However, there is no real evidence that vegetative heterokaryons can be formed from cultures of opposite mating-types. The experiments to be described in this paper were made to investigate this point on the suggestion of Dr. B. O. Dodge. The results are discussed in relation to the mating-type factors and sexual reproduction in *N. crassa* and *N. tetrasperma*.

MATERIALS

Preliminary experiments were made on a wild type perithecium-forming strain obtained from Dodge under the name *N. crassa*, "Abbott," and also on combinations involving two mutants which were very similar in appearance, namely the mutant "*crisp*" described by Lindegren (1936) and a new mutant "*star*" on the same chromosome as *crisp* and the mating-type factors. Further experiments were made on two pairs of mutants *X–8*, *X–9*, *X–10*, and *X–11*. These mutants were all obtained in the first place by X-radiation of microconidia of the *fluffy* strain. The mutants were then crossed with *wild type* and from the resulting ascospore cultures mutant cultures of *A* and *a* mating-types were selected. Mutants were chosen for their reduced growth-rate, since it was believed that this would favor the production of heterokaryons, and for their dissimilarity to each other.

X–8 and *X–9* were distinguished from *wild type* and *fluffy* by their greatly reduced growth-rates and special habit of branching, and they could

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be readily distinguished from each other because *X–9* showed clustered branching at the ends of the hyphae whereas *X–8* did not.

X–10 and *X–11* also showed reduced growth and characteristic branching. In plate cultures the growth was of a rather spreading colonial type. Both mutants had an intense pink coloration when exposed to the light. The *X–11* mutant gave a more reduced compact type of growth than *X–10*, as illustrated in figures 2 and 3.

EXPERIMENTS AND RESULTS

N. crassa, "Abbott." A mass transfer was made from the original tube which contained perithecia. Macroconidia, which are usually multinucleate, from this subculture were spread thinly on a plate of potato-dextrose agar. On germination, 30 single macroconidia were isolated into tubes of potato-dextrose agar. One of these failed to grow, the remaining 29 cultures did not form perithecia. When tested with *A* (Lindegren's +) and *a* (Lindegren's –) strains, these cultures all reacted as *a*, giving perithecia with the *A* strain but not with the *a* strain. This strain therefore gave no evidence of the perpetuation of vegetative "bisexual" heterokaryons. It seems that the *a* component of the original culture may be more vigorous than the *A* component, but the experiment would have to be repeated several times with similar results before this could be taken as established.

Crisp and Star Combinations. *Crisp a* and *star a* inoculated together gave cultures which are *wild type* in appearance and therefore, presumably, heterokaryons. *Crisp a* and *star A* were inoculated together many times, the cultures usually remaining mutant in appearance. On one occasion, however, the combination gave a culture which appeared to be of the *wild type*. Eighteen hyphal tip isolations were made from this culture and all were *wild type* in appearance. From one of these hyphal tip isolates, 40 macroconidial isolations were made, all of which were phenotypically *wild type*. It was thought therefore that this was a true heterokaryon involving *crisp a* and *star A* nuclei. However, 31 of the macroconidial isolates were tested with *A* and *a* strains and they all gave the *A* reaction only. This led to the suspicion that the apparent heterokaryosis was actually the result of a mutation in the *star A* nucleus causing it to give a mycelium of normal appearance. The mutated *star A* nucleus probably results in a more vigorous mycelium than that of *crisp*, thus accounting for the absence of *crisp* among the original hyphal tip isolates. This suspicion was verified by an examination of subcultures from the original *star* culture which also appeared normal, and by the results of crosses between the reverted *star* and normal. These gave some *star* segregants, indicating that either the mutation modifying *star* to normal was not a reversion at the *star* locus but was a change at a different locus modifying the expression of *star*, or that the reverted *star* strain used in the

cross with normal was a heterokaryon containing some *star* nuclei. No evidence for the production of "bisexual" heterokaryons was obtained from these experiments.

Since macroconidial and ordinary hyphal tip isolations had given no evidence of heterokaryosis involving nuclei of opposite mating-type, it was determined to make hyphal tip isolations from outgrowths of young perithecia, following the method of Dodge (1935).

X–8 A and X–9 A Combinations. *X–8 A* and *X–9 A* gave a culture resembling that of standard *fluffy*. Twelve hyphal tip isolates were all *fluffy* in appearance. When uninucleate microconidia of two of these hyphal tip cultures were plated out and isolated, the following results were obtained. The first culture yielded 119 cultures of *X–8* type, 113 cultures of *X–9* type, and 1 normal. The second culture gave 131 cultures of *X–8* type, 205 cultures of *X–9* type, and 2 normal. The three exceptional normal cultures when subjected to microconidial analysis gave *X–8* : *X–9* types in the following ratios, 39 : 46, 26 : 23, and 8 : 33. They probably resulted from the accidental picking up of two germinating microspores at once or from the occurrence of an occasional binucleate spore. These experiments show that combinations of *X–8* and *X–9* mutants readily form vigorous heterokaryons, when they are of the same mating-type, *A*.

X–8 a and X–9 A Combinations. *X–8 a* was inoculated together with *X–9 A*, with no apparent increase in vigor. The culture was allowed to form perithecia. Individual perithecia were isolated, the pieces of adhering mycelium were removed from them, and they were transplanted to petri dishes. Hyphal tip isolations were made from the hyphae which had the appearance of growing out from these perithecia. Forty-eight such isolations were made, of which 18 proved to be of the *X–8* type and 30 of the *X–9* type. When tested with standard *A* and *a* strains, the *X–8* type gave only the *a* reaction and the *X–9* type only the *A* reaction.

This experiment was repeated but the cultures obtained from each perithecium were kept separate. Eight perithecia were analyzed, the number of isolates from the different perithecia being 6, 7, 8, 14, 14, 14, 17, and 27, respectively. All the cultures were of one type, namely the *X–9* type. This evidence indicates that hyphae of only one mating-type are concerned in the formation of individual perithecia. The fact that only one type of perithecium was found in the second experiment may possibly be due to the earlier selection of perithecia in the second experiment. It is possible that *X–8* tends to form perithecia later or less readily than *X–9*. This would be in accordance with the reduced vigor of *X–8*.

Combinations Involving X–10 and X–11. A further series of experiments was made in order to test the hypothesis suggested by the results of

the last experiment, that perithecia are made up of hyphae of one mating-type only. For these experiments mutants *X–10* and *X–11* were used. Figure 1 shows tube cultures of *X–10 a*, *X–10 A*, *X–11 a*, (*X–10 a* + *X–11 a*), and (*X–10 A* + *X–11 a*). The cultures were all inoculated at the same time into tubes of potato-dextrose agar. The combination of *X–10 a* and *X–11 a* clearly shows heterokaryotic vigor, whereas no such effect is shown by the *X–10 A* + *X–11 a* combination.

The mutants *X–10 A* and *X–11 a* were then inoculated on to a petri dish of cornmeal agar, which is favorable for perithecium production. Two inoculations of *X–10 A* were made at one side of the petri dish and two of *X–11 a* at the other. The mutants grew separately until they met. Perithecia were formed at the line of junction and spread out about 1 cm. on either side of it.

Single perithecia were isolated as before and the pieces of adhering mycelium were carefully removed by using fine needles and washing on a slide in sterile physiological saline solution. The perithecia were then transplanted to plates of plain 3 per cent agar. The use of plain agar induces a thin spreading growth and so facilitates the removal of single hyphal tips. After two days the cultures developing from the perithecia were examined. They were seen to be of two distinct types, as illustrated in figures 2 and 3. One type of perithecium, taken from the *X–10* side of the culture, gives rise to a rather loose colony, the other, taken from the *X–11* side, to a very dense, compact type of colony. When two perithecia taken from opposite sides of the culture are put together, the hyphae appear to grow separately (figs. 4, 5).

Altogether 54 perithecia isolated proved to be of the *X–10* type and 28 of the *X–11* type. The greater number of perithecia of the *X–10* type as compared with the *X–11* type is probably to be correlated with the more vigorous, spreading habit of the *X–10* type as compared with the *X–11* type.

As a further test, 50 hyphal tip cultures were made from the *X–10* type of perithecium, 10 from each of 5 perithecia. Similarly, 50 cultures were made, 10 each from 5 perithecia of the *X–11* type. However, in this case the growth was too compact for single-tip cultures to be made, so small inoculum cultures were made instead. The 50 cultures from the *X–10* type of perithecium proved to be of *X–10* type in appearance and the 50 cultures from the *X–11* perithecia were of *X–11* type. None of these cultures had produced perithecia three months after inoculation.

A single tip from an *X–10* perithecium was inoculated together with a small inoculum from an *X–11* perithecium into each of 10 tubes. After 2 months, perithecia were found in 9 of these tubes. In the remaining tube, only the *X–11* type appeared to have grown.

Furthermore, eight cultures from each of 5 different perithecia of the *X–10* type, when tested with *A* and *a* strains, gave the *A* reaction only, while

nine cultures from each of 5 different *X–11* perithecia gave the *a* reaction only.

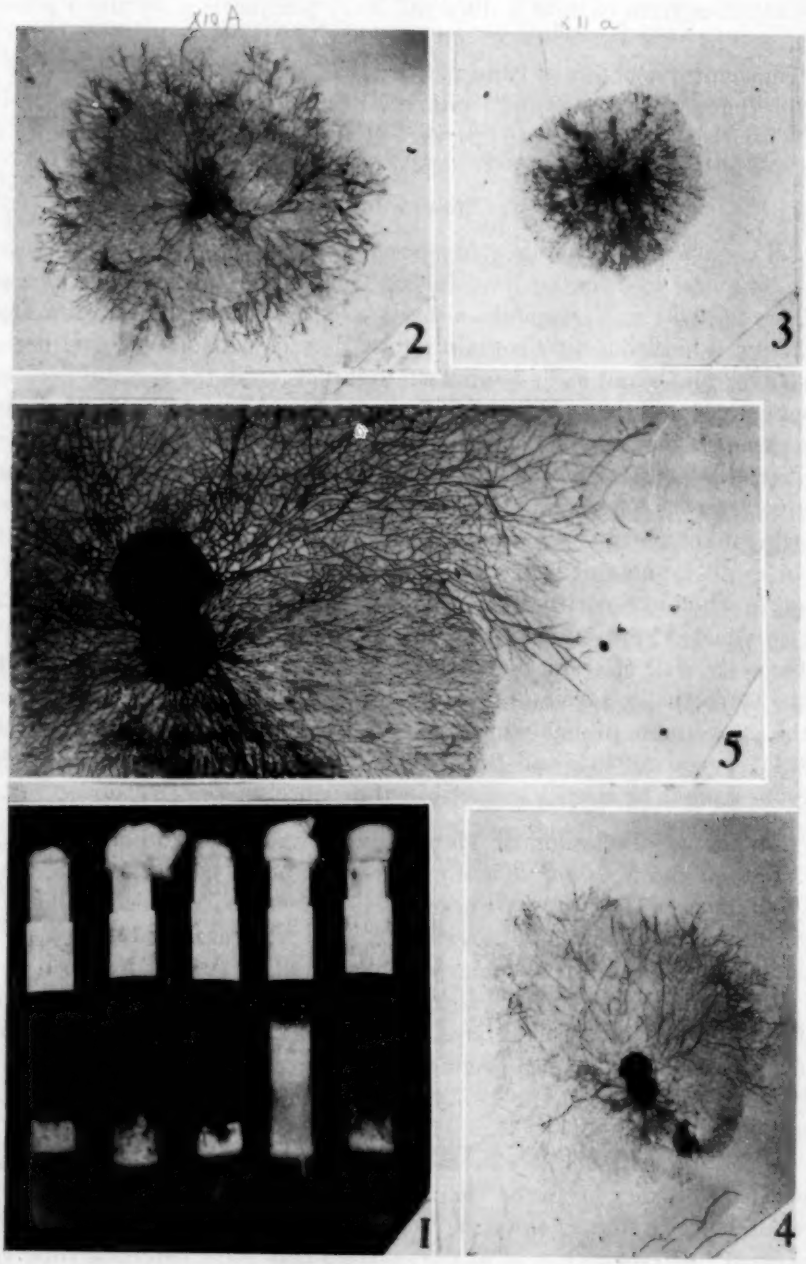
Thus cultures obtained from perithecia of the *X–10* type were all of the *X–10* type in appearance and "unisexual" of the *A* type. Cultures derived from perithecia of the *X–11* type were of the *X–11* type in appearance and "unisexual" of the *a* type.

DISCUSSION

All my attempts to obtain evidence of vegetative heterokaryosis involving *A* and *a* nuclei in *N. crassa* have failed. This is in accordance with the work of Dodge (1935) on *N. sitophila*, a closely related heterothallic species. Dodge transferred blocks of agar containing fertilized but immature perithecia to fresh agar plates and made hyphal tip isolations from the resulting cultures. One hundred and thirty-six such isolates produced no perithecia. He did not test them for mating-types. In the present experiments, heterokaryosis was favored by the use of mutants having a low growth-rate, which were known to give vigorous heterokaryons when of the same mating-type. Nevertheless no evidence of vegetative heterokaryosis involving nuclei of opposite mating-types could be obtained. Moreover, the results from the *X–10* and *X–11* cross, in which the perithecia of the two types could be distinguished (figs. 2, 3), indicate that mycelia of only one mating-type enter into the formation of the body wall of a single perithecium. We must conclude therefore that in the heterothallic species, the association of the two types of nuclei occurs in the ascogonium, probably by way of the trichogyne as described by Colson (1934), Dodge (1935), and Backus (1939). Vegetative "bisexual" heterokaryons cannot be readily induced in these species.

Contrasting Behavior of Heterothallic and "Homothallic" Species. In *N. crassa* and *N. sitophila* mating depends upon a pair of factors, the + – or *A a* factors, which segregate at meiosis in the ascus. Meiosis is followed by a third mitotic division and as a result 8 ascospores are produced, 4 of type *A* and 4 of type *a*. In the secondarily "homothallic" species, *N. tetrasperma*, the *A a* factors are present and segregate at meiosis as in *N. crassa* but nuclei of the *A* and *a* type are associated in the young ascospore so that normally four "bisexual" spores are produced. "Heterothallic" lines which contain only one type of nucleus can be obtained by conidial isolations from the normal "homothallic" type.

As has been mentioned in the introduction Dodge (1942) produced vigorous heterokaryons in the "homothallic" species *N. tetrasperma*, whether he combined two lines of the same or of opposite mating-types. The fact that "bisexual" heterokaryons in *N. tetrasperma* may show heterokaryotic vigor renders less plausible the suggestion of Beadle and Coonradt (1944) that the lack of heterokaryotic vigor in "bisexual" combinations of *N. crassa* is due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to a direction of the energies of the plant towards sexual reproduction rather than vegetative vigor. Dodge (1935) found that hyphal tip isolations made from outgrowths from small blocks of agar bearing young perithecia obtained by mating "unisexual" lines of *N. tetrasperma* were uniformly "bisexual." Although one can often isolate from such "bisexual" mycelia single tips which will be "unisexual," it seems that the difference between the so-called "homothallic" species and the heterothallic species of *Neurospora* is not merely a question of the mechanism of spore formation, but rather that nuclei of opposite mating-types are normally associated at all stages in the "homothallic" species, whereas in the heterothallic species they are kept apart until the time of sexual reproduction, as suggested by Dodge (1935).

"Bisexual" Heterokaryons in N. crassa. Lindegren (1934a) found that three out of five strains isolated from nature formed perithecia with both *A* and *a* strains, although they were self-sterile. These strains, which were of the conidial *wild type*, were crossed with tester strains of the *fluffy* type. A number of asci from these crosses were dissected and it was found that certain asci contained ascospores which gave rise to *wild type* and *fluffy* cultures in the expected 1:1 ratio, but other asci gave rise only to *wild type* cultures. The latter are supposed to be due to self-fertilization and are taken to indicate that the wild type is actually dikaryotic for the mating-type factors. This assumption is borne out by the fact that one "bisexual" strain showed somatic segregation, giving subcultures with one mating-type reaction only. Lindegren accounted for the self-sterility of the wild type cultures by assuming that they possessed genetic factors for heterokaryon self-sterility. Some support for this assumption is to be found in the results of combinations between some of the segregates from the original crosses. It was found that some of these combinations did not form perithecia, although known to be of opposite mating-types, because of their reaction with tester *A* and *a* lines. Lindegren suggests that "bisexual" heterokaryons arise in nature by the anastomosing of hyphae containing *A* nuclei with hyphae containing *a* nuclei and that in certain conditions natural selection will act in favor of self-sterile heterokaryons. The evidence of the present experiments

Explanation of figures 1–5

FIG. 1. 4-day cultures on potato-dextrose agar of *X–10 a*, *X–10 A*, *X–11 a*, (*X–10 a* + *X–11 a*), and (*X–10 A* + *X–11 a*). Heterokaryotic vigor is shown by the combination involving two mutants of the same mating type *a* but not by the combination involving mutants of different mating type. FIG. 2. 4-day colony from young perithecium of the *X–10* type. ×8. FIG. 3. 4-day colony from young perithecium of the *X–11* type. ×8. FIG. 4. 4-day colony from young perithecia of the *X–10* and *X–11* types. ×8. FIG. 5. Part of figure 4 showing the mycelia of *X–10* and *X–11* type growing side by side. ×25. Figures 2–4 were obtained by growing the colonies on cellophane strips (Fleming & Smith 1944) on plain agar, and mounting on microscope slides for photographing. Figure 5 was taken from the colony illustrated in figure 4 after it had been fixed in formal-acetic, stained with chlorazol black and mounted in gum chloral. Photograph and photomicrographs by E. Ashby.

and of the work of Köhler (1930) indicates that "bisexual" heterokaryons are not normally formed in this way. If the "bisexual" heterokaryons found by Lindegren originated by mycelial fusion they must have possessed factors permitting the vegetative association of *A* and *a* mycelia not present in the lines studied in these experiments. It is possible that these heterokaryons arose from "bisexual" spores. Lindegren (1934b) reported an exceptional ascus in *N. crassa* which had four large ascospores and four small "akaryotic" spores. The large spores gave rise to self-fertile cultures. Single conidial isolations were made from these cultures and, while most of these were "bisexual," some were "unisexual" as the result of somatic segregation. This somatic segregation proves that the cultures were heterokaryotic for the mating-type factors. Ascospores from self-fertilized perithecia gave heterothallic "unisexual" cultures.

Moreau and Moruzi (1932a, b, c) also obtained cultures from giant ascospores of *N. sitophila* which were "bisexual" and self-fertile. They do not report the occurrence of somatic segregation in these forms, but ascospores from the self-fertilized perithecia gave heterothallic cultures. The "bisexuality" in these instances also was almost certainly due to the fact that the cultures were heterokaryotic for the *A a* factors.

These "bisexual" heterokaryons arising in experimental material differ from the natural races investigated by Lindegren in being self-fertile. Since somatic segregation was observed in one case in which single conidia were isolated, it is possible that the fertility is due to a reaction between the heterokaryon and segregated homokaryotic mycelia. The author has also had some single-ascospore cultures which give both *A* and *a* reactions and appear to be heterokaryotic for these and other factors. These cultures form selfed perithecia but rather sporadically, as one would expect if perithecial formation is dependent upon the somatic segregation into one or other of the "unisexual" types.

Hormones and Sexual Reproduction in Neurospora. Moreau and Moruzi (1931) claim that perithecial formation in *N. sitophila* is caused by the stimulation of the mycelium of one mating-type by a hormone produced by the other mating-type and may be independent of the fusion of two nuclei of opposite mating-types. They inoculated one mating-type into one arm of a U-tube containing cornmeal agar and the opposite mating-type into the other arm. Ripe perithecia were produced in one arm of the tube and large sclerotia (incipient perithecia) in the other. The authors describe experiments by which they claim to show that mycelia do not pass from one arm to the other and therefore they attribute the formation of perithecia to the stimulation of a hormone. Dodge (1932) and Aronescu (1933, 1934) repeated these experiments and found that one type of mycelium grows into the other arm if air pockets are formed. Aronescu also dissected and grew the asco-

spores from single asci and in every case found a segregation of the two mating-types in a 1:1 ratio. There is reason to suppose, therefore, that the U-tube method does not afford a satisfactory means of keeping the two types of mycelium apart after air pockets develop in the culture. Lindegren (1934a) has suggested that the line producing perithecia in Moreau and Moruzi's experiments is a self-sterile "bisexual" heterokaryon and that hormones produced by the second line neutralize the effect of the self-sterility factors. More recently Moreau and Moreau (1938a, b) have made further experiments which appear to show the presence of a hormone concerned with perithecium production in *N. sitophila* and *N. tetrasperma*. Cultures of one mating-type were grown on cornmeal agar in a petri dish. When the cultures were well grown the agar was sterilized by heating with steam without pressure for 10 minutes, or by treating with ether or chloroform vapor. Afterwards samples were taken from the agar and incubated, and at the same time the agar was inoculated with the opposite mating-type. The samples showed no growth, indicating that the original mycelium had been killed by the treatment, and the mycelia of the opposite mating-type showed perithecial formation, though somewhat irregularly. This is taken to indicate that perithecial formation is stimulated by a hormone not completely stable at 100° C. Unfortunately Moreau and Moreau do not report on the mating-type reactions of the cultures coming from such perithecia. If the asci result from self-fertilization of a truly "unisexual" type they should produce spores of one mating-type only. If however they are the result of accidental contamination with the opposite mating-type or of the effect of hormone action on a dikaryotic potentially "bisexual" type, they should give cultures of both mating-types. It is conceivable that a potentially "bisexual" type might give one mating-type reaction only but when selfed it should give spores of both types.

In the absence of evidence that the ascospores produced by selfing induced by hormone action are of one mating-type only, we must continue to believe that the sexual reaction involves the fusion of two nuclei of opposite mating-types. On the view that hormone action in itself is sufficient to induce ascus formation in a "unisexual" mycelium, occasional selfed perithecia would be expected to occur in ordinary crosses. Thousands of asci have been dissected by the Lindegrens and others without such perithecia having been found, except in lines which could be shown to be heterokaryotic for mating-type.

Sexual Reproduction as the Result of Hormone Action and the Fusion of Nuclei of Opposite Mating-Type. There is considerable evidence that sexual reproduction in *Neurospora* depends upon the fusion of two nuclei of opposite mating-types, and no conclusive evidence that it can occur without this. Nevertheless, there are certain reasons for supposing that hormones may play a part in sexual reproduction.

Single-spore cultures of different mating-types, if kept isolated, can produce sclerotia or incipient perithecia. The extent to which these are produced depends upon the strain of fungus and the cultural conditions. Both mating-types can produce these sclerotia under suitable conditions. Nevertheless they seem to be produced much more rapidly in the presence of the opposite mating-type, which may indicate that one mating-type secretes a substance stimulating incipient perithecium formation in the other.

The behavior of self-sterile "bisexual" heterokaryons, which when brought into contact with a heterothallic line produce selfed as well as crossed asci, is best explained by assuming that the self-sterility is removed by the stimulus of substances produced by the heterothallic line. The experiments of Moreau and Moreau also appear to indicate the occurrence of a hormone connected with perithecium formation.

It seems feasible that the formation of functional perithecia is dependent upon a hormone which can only be produced by "unisexual" lines or which is dependent for its formation on the interaction of lines of opposite mating type. On this hypothesis the occurrence of selfing in a "bisexual" strain is believed to be due to the somatic segregation into one or more "unisexual" types, leading to perithecial formation. "Bisexual" lines that are self-sterile are believed to be so because of the suppression of somatic segregation in these lines. Self-fertilization may be induced in such types by bringing them into contact with "unisexual" lines of independent origin, with a consequent stimulus to perithecium formation.

If perithecium formation is dependent upon the presence of a "unisexual" line or the interaction of lines of opposite mating-types, it is clear that the production of somatic heterokaryons involving nuclei of opposite mating-types must not take place if sexual reproduction is to occur. This phenomenon by which heterokaryons may be readily formed by mycelia with nuclei of the same mating-type but not between those of opposite mating-type is probably very widespread. Zickler (1934) was the first to prove that in certain heterothallic ascomycetes the sterile part or framework of a perithecium is not formed by an intermingling of hyphal branches from both mating-types grown in the culture. On the contrary, if a perithecial primordium arising, for example, from a reddish-brown mycelium of *Bombardia lunata* is spermatized with spermatia from a greenish mycelium of opposite mating-type, the mature perithecium will be reddish-brown; and vice versa. Hansen and Snyder (1943) describe two lines in *Hypomyces solani* f. *cucurbitae*, one with red, the other with white perithecia. In crosses between these two lines the perithecia are either red or white, indicating that only one mating-type goes to form the framework of a single perithecium. Vandendries (1934) described an apparently similar type of behavior in the basidiomycete *Lenzites betulina* (L.) Fr. In this species, there are four

mating-types depending upon two factor-pairs, and there is a "barrage sexuel" between *b* and *b′* and independent of *a* and *a′*.

The capacity to form heterokaryons may not be determined by the mating-type factors alone. Indeed one case was observed in which two mutants of the same mating-type would not form a heterokaryon when grown together. It is possible that the reverse phenomenon, by which modifying factors permit the vegetative association of lines of opposite mating-type, may also occur. Such modifying factors would account for the behavior of the self-sterile "bisexual" heterokaryons described by Lindegren. Somatic segregation in such a type would be followed by re-fusion so that perithecium formation would not occur. The effect of somatic segregation would also be neutralized if the segregants had a lower growth-rate than the heterokaryon. However, this explanation would not account for the "sterile" combinations between lines derived from the "bisexual" strains, whereas vegetative fusion would.

In the heterothallic species, therefore, sexual reproduction seems to involve at least two types of reaction. The production of functional perithecia seems to be dependent upon the presence of "unisexual" lines, possibly of two types, and the actual process of fertilization depends upon the fusion of two nuclei of opposite mating-type in the young ascus. Sexual reproduction therefore depends upon an interaction between *mycelia* of opposite mating-type, in which hormone action may be concerned, as well as upon the fusion of nuclei of opposite mating-type. In the "homothallic" forms, the interaction between mycelia of opposite mating-types has been eliminated. The "bisexual" mycelium forms perithecia without any external stimulus. The way in which the secondary homothallic type has evolved from the heterothallic one is a problem that requires further investigation.

Beadle and Coonradt (1944) suggest that the phenomenon of heterokaryosis is a step in the evolution of a sexual reproduction. This assumes that the *A a* factors are true sex factors. However, it seems more probable that *Neurospora* is hermaphrodite and the *A a* factors are more closely related to incompatibility factors (Sansome & Philp 1932) since both types produce ascogonia, the female sex organs, and microconidia which may correspond to spermatia. A consideration of the work of Zickler (1934, 1937) on *Bombardia lunata* and that of Hansen and Snyder (1943) on *Hypomyces solani* f. *cucurbitae* is in confirmation of this view. In *Bombardia* what appear to be true sex factors are inherited independently of the mating-type factors. The primitive sexual condition was probably an 8-spored homothallic type, heterothallism being a derived condition. The present work indicates that heterokaryosis could not have been a step in the evolution of the heterothallic system, since a suppression of heterokaryosis is associated with the mating-type factors. Heterokaryosis is probably a fairly primitive condition since

it is of widespread occurrence. On the other hand, it may have evolved independently in different groups, especially since it is a very favorable condition. It would appear to be the ideal condition for a multinucleate, relatively undifferentiated organism because of its extreme flexibility, giving the possibility of rapid adaptation to different conditions. The heterothallic species such as *Neurospora crassa* are able to form heterokaryons within each mating-type and under suitable conditions sexual reproduction takes place between opposite mating-types. In the secondary "homothallic" types such as *N. tetrasperma* the *A a* nuclei are associated throughout the life history and there is no restriction on heterokaryosis.

SUMMARY

The following evidence indicates that heterokaryons are not normally formed involving the *A* and *a* factors in *N. crassa*. The *A* and *a* nuclei appear to be kept apart until the time of sexual reproduction and are probably brought into association by way of the trichogyne.

X–8 A and *X–9 A* cultures readily form heterokaryons with greatly increased vigor. *X–8 a* and *X–9 A* combinations remain mutant in appearance. Hyphal tip isolations from perithecia of this combination gave 18 cultures of *X–8* type and 30 of *X–9* type. In a second cross, from which eight perithecia were isolated, 101 hyphal tip cultures all of *X–9* type were obtained.

In a similar experiment involving two further mutants, *X–10* and *X–11*, it was found that the colonies formed by individual perithecia could be classified as *X–10* or *X–11* in type. Fifty-four perithecia of *X–10* type and 28 perithecia of *X–11* type were isolated. Hyphal tip isolations from the *X–10* type perithecia were *X–10* type in appearance, and small inoculum transfers from the *X–11* type of perithecium were *X–11* type in appearance. All such isolations were "unisexual" and when tested the *X–10* type were *A* and the *X–11* type were *a* in mating-type reaction.

These results are discussed in relation to the behavior of the "homothallic" species *N. tetrasperma* and to theories on the role of hormones and nuclear fusion in sexual reproduction in *Neurospora*.

The experiments described in this paper were commenced at the Department of Genetics of the Carnegie Institution of Washington, Cold Spring Harbor, New York, and finished at the Department of Cryptogamic Botany of the University of Manchester. The author wishes to thank the directors and staffs of these departments for their help and encouragement, and Dr. B. O. Dodge and Prof. F. J. Ryan for critical reading of the manuscript and helpful suggestions.

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SELF-STERILITY IN "BISEXUAL" HETEROCARYONS OF NEUROSPORA

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In the work on heterocaryotic vigor in *Neurospora tetrasperma* (Dodge, Schmitt & Appel, 1945) we found at times that certain factors must be interfering with the orderly development of perithecia in cultures of some of the f_1 heterocaryotic segregant races. If we consider merely the obvious effects, there appeared to be at least two or three kinds of sterility. (1) Many perithecia developed to full size in the culture, but no asci ever arose in them. The full functioning of the mating-type factors had been blocked, but not until the framework of the perithecium had been completed. The sterility factors became effective first at the critical stage of sexual reproduction, namely, at the formation of ascogenous hyphae. It might have been a simple matter to eliminate these interfering factors, but our problem was to follow the complementing factors for heterocaryotic vigor as they were handed down by the parent races along with the sterility factors regardless of how much trouble the latter gave us when it came to genetic considerations. (2) Some of our bisexual f_1 heterocaryotic races grew vigorously in culture, but only a very few perithecia made their appearance. Races in this group were of two kinds. The few perithecia that were finally developed in certain cultures delimited many ascospores, while no asci were formed in perithecia of other races. (3) No perithecia were ever formed in cultures of certain races which were later proved to be heterocaryotic for mating type factors. It was believed that the operation of the "sterility" factors in groups (2) and (3) above was merely one of degree of the sterility affected, rather than a difference in the nature of the factors which limited the number of perithecia in a culture.

Experiments with breeding races of *N. sitophila* which will be described later seem to afford one possible explanation for the sterilities in groups (2) and (3) of *N. tetrasperma* noted above. The same explanation may apply to the "bisexual" self-sterile heterocaryotic races of *N. crassa* discussed by Lindegren (1934) and by Sansome (1946), and to certain cross-sterilities between two unisexual races even though they are of opposite mating types.

Aronescu (1933), working in this laboratory with certain non-conidial races of *N. sitophila*, made crosses between two races, one of which developed large numbers of incipient perithecia, and the other of which produced very few if any of these receptive structures. She isolated the eight spores from some of the F_1 asci and proved for the first time that the factors controlling

the relative abundance or paucity of incipient perithecia produced by individual races are segregated at meiosis. She could not carry on this work to its theoretical conclusion. That is, she did not mate or grow together in culture two races of opposite mating-type, each of which produced by itself an abundance of incipient perithecia, and then by contrast grow together two races of opposite mating-type, neither of which developed by itself any of these receptive bodies. This the writer has now done. The results of such matings, as well as brief studies made on a few "wild type" conidial races from bread from various localities, will be reported here.

The races employed by Aronescu were obtained by mating two non-conidial races of *N. sitophila* originally described by the writer (1930). She did not give the full pedigree of the strains she used but it is certain that they were both direct descendants from the mating 56.2 × 56.6. Both these races originally produced an abundance of incipient perithecia. Backus (1939) employed them in his excellent work on "conidiation." The writer has carried these races in culture for 17 years. Race 56.2, mating-type *a*, has not changed materially, but race 56.6, mating-type *A*, no longer produces incipient perithecia. We shall from now on refer to these receptive bodies as "protoperithecia," a good term coined by Buller. The symbol *P* will be used to designate a race that produces by itself protoperithecia, and *p* will be used to designate a race that produces few if any of them.

Ascospores from the mating 56.6 × 56.2 were sowed on plates of Difco potato-dextrose agar and then given the heat treatment. Some 200 or more single germinating ascospores were isolated and grown in tubes. These cultures were observed frequently for several weeks to determine which ones produced protoperithecia and which ones did not. In the meantime the mating-type, *A* or *a*, of each of 152 of these f_1 segregant races was determined. The tester races used were 56.2, mating-type *a*, and f_1 races Nos. 36, 39, 44, and 50, which were mating-type *A*. All five testers produced many protoperithecia. The results of these tests were as follows: 73 were mating-type *A* and 79 were mating-type *a;* 72 were *P*, producing protoperithecia, and 80 were *p*, producing few if any protoperithecia; or 34 *PA;* 38 *Pa;* 39 *pA;* 41 *pa*, that is, 77 old combinations to 75 new combinations. There was no evidence of linkage.

From the 80 races which did not seem to produce protoperithecia we chose thirty-four races at random. Fourteen of these happened to be mating-type *A* and twenty were type *a*. These were divided into two groups each of which included seven of mating-type *A* and ten of type *a*. Within each group the races were grown together in pairs in all combinations of the *A* and *a* types, and checks with the parent races 56.2 and 56.6 were also included. The cultures of the mated races were observed for several weeks or until they dried out. Not one of the 140 mixed cultures produced peri-

thecia. No *pA* race fruited with a *pa* race, and no *pa* race fruited with the tester 56.6, which is *pA;* but all the *pA* races so far as tested fruited with the tester 56.2, which is *Pa*. Zickler (1934), working with *Bombardia lunata*, indicates by his diagrams that he was unable to obtain perithecia when he cross-spermatized two races neither of which produced protoperithecia. He does not state, however, that he also grew his two races together in the same culture.

So far as our experiment goes we must believe that either the one or the other of the two races mated must produce protoperithecia if fertilization leading to production of perithecia is to be consummated. We found no evidence that a race *pA* could stimulate the formation of protoperithecia by a race *pa*, or vice versa. This does not mean, however, that a race which is producing a *few* protoperithecia in a culture may not be induced to produce additional protoperithecia, as Sansome (1946) also appears to believe, by a change in the medium, by the addition of hormones, by the introduction of some other race into the same culture, or by the application of microconidia or monilioid conidia. It is clear that whenever a race which has been proved to be heterocaryotic for mating-type factors *A* and *a* fails to produce perithecia, it may well be that neither component of the heterocaryon could produce protoperithecia if grown by itself.

"DOMINANCE" IN MIXED CULTURES

Heretofore whenever we have grown races 56.2, *Pa*, and 56.6, *pA*, together in culture, protoperithecia always developed. It would be curious reasoning to call this a dominance of *P* to *p*, or to say that protoperitheciumformation is a dominant character, especially as we have not yet proved that a mingling of these two mycelia in culture ever results in heterocaryosis. This work should be carried on further in the light of Sansome's report (1946) that in the obligately heterothallic species, *N. crassa*, one can readily obtain heterocaryotic races by growing two different races together provided these races are of the *same* mating-type, but not when they are of *opposite* mating-type. The writer, working with the same "Abbott" race of *N. crassa* has fully confirmed the latter part of her claim. The first part would also be confirmed if we grew our race 56.6, which is *pA*, with race f_1 200, for example, which is *PA;* or grew race f_1 210, *Pa*, with race f_1 280, *pa*. In both cases protoperithecia will be formed in the cultures but one will not know whether or not the mycelium is really heterocaryotic until both components, either *PA* and *pA* or *Pa* and *pa*, can be isolacted from a mycelium derived from a single-tip isolate from the suspected heterocaryon. Would it not be illuminating to find that the heterocaryons, either (*Pa* – *pa*) or (*PA* – *pA*), would not produce protoperithecia in culture? It would be difficult to refute the argument that *p* is dominant to *P* and therefore we

should transpose our symbols. The writer was much disturbed by the results of certain plate culture experiments which showed a sort of composite effect of a type of "dominance" such as just hypothesized above.

"DOMINANCE" IN MULTI-SPORE CULTURES

The plate cultures from which the 200 or more germinating ascospores had been isolated, as noted earlier, were set aside for several weeks. Many germinating ascospores still remained in each plate and these spores had continued to grow. There were, therefore, probably two or three hundred different f_1 mycelia in each plate. Approximately 50 per cent of these mycelia must have carried the factor *P* for protoperithecia and the rest must have carried *p*. The mating-type factors, *A* and *a*, must also have been equally represented. Theoretically, protoperithecia and later perithecia should have developed in these plates. Such was not the case, however; none of these bodies was found in the cultures even after several weeks. Believing that some faulty technique or culture conditions were responsible for this apparent "dominance" of the factor *p*, we repeated the experiment three times sowing as before many ascospores on plates of potato-dextrose agar. After heat treatment it was seen that many ascospores had germinated, but none of them was removed from the plates. The results were the same as before. No protoperithecia and no ascocarps ever developed in any of the eight different plates, although in each there must have been a hundred or more mycelia of each mating-type and fifty per cent of them would, if isolated and grown by themselves, have produced protoperithecia. Since no receptive bodies were ever developed in the plates no perithecia could be expected to form. The failure of such multispore cultures to produce protoperithecia was probably merely a reaction to unfavorable culture conditions brought about by the excessive dosage of germinated ascospores. It has nothing to do with dominance. A similar experiment but made with a different culture medium might have resulted quite differently.

The writer is indebted to Mr. Charles Beall for isolating over 200 germinating f_1 ascospores from the mating 56.2×56.6. He recorded that of the 162 f_1 races studied 107 were judged to produce few if any protoperithecia. Observations made after his cultures had dried out, however, proved that quite a few of these cultures had actually good protoperithecia which had been overlooked. Only 53.2 per cent of those checked failed to produce receptive bodies. In both sets of our experiments the numbers of races that failed to produce protoperithecia were somewhat in excess of those that developed these bodies.

Aronescu (1933) inoculated the medium in one arm of a U-tube with two races of *N. sitophila*, one of which was conidial and the other non-conidial, the two races being in each case of opposite mating-types. Perithecia soon

developed in the first arm at the point of inoculation. In due time mycelial hyphae appeared at the surface of the medium in the second arm. In both U-tubes figured in her plate 14, the mycelia that finally appeared in the second arm bore conidia. Was this a proof of heterocaryosis attained, and of the dominance of *C* to *c*? The answer is no. She proved that only *one* of the two mycelia used in the 86 experiments ever arrived in the second arm. The one that got started first or had the faster inherent growth rate was the one that "dominated" the situation. Aronescu did not in any case inoculate the one arm with non-conidial and conidial races both being of the *same* mating types. She did prove, however, that heterocaryosis was not consummated in any case where the two races were of *opposite* mating-types.

Certain other experiments involving tests for mating-types indicate how badly one can be misled by superficial appearances when judging heterocaryotic conditions and so-called dominance in mixed cultures. A few examples will illustrate this point. Voth 2, Voth 5, Wall, Sands, HSS f_1, 1, 5, 8, 15, and 24 were all conidial, mating-type *A*. Voth 4, HSS f_1, 17, 22, 28, and 33 were all conidial, mating-type *a*. Each of these races was grown separately in mixed culture with race 56.2, non-conidial, mating-type *a*. The mixed cultures Voth 4 + 56.2 ($a + a$) and Wall + 56.2 ($A + a$) produced many characteristic orange-colored conidia, while all the other mixtures with 56.2 were albinistic and practically non-conidial. All mixtures of opposite mating-types produced perithecia, proving that both races in each case grew well. The races in mixtures 56.2 + HSS f_1, 33 and 56.2 + HSS f_1, 17 were of the same mating-type and so both mixtures could have been heterocaryotic. Some would claim that *c* was dominant to *C*. In mixture 56.2 + Voth 4 ($A + a$) however the *C* was "dominant" to *c*.

PROTOPERITHECIA ON WILD-TYPE MYCELIA

Shear and Dodge (1927) noted that certain races of *Neurospora* produced numbers of little "sclerotia" or "bulbils," as they called them, while other races produced very few of them. The following year the writer (1928) said: "Mycelia of species of *Neurospora* produce coiled structures which very likely are morphologically equivalent to sex organs." It was not until later (1932, Dodge & Swift 1933) that it was proved experimentally that the "sclerotia" of *Neurospora* are receptive structures. Since then we have been careful to keep as our tester races only those that develop incipient perithecia when grown alone. Whether or not races being tested produced them was usually not recorded.

The failure of race Dwarf 16 (Dodge, Schmitt & Appel 1945) to produce protoperithecia could be due to the blocking effects of the dwarf factor *v*. This same dwarf race does not produce the monilioid conidia in culture, but it has been proved genetically that it carries the factor *C* for conidia. This

factor *C* is also blocked, or prevented from expressing its effects, by the dwarf factor *v*.

During the past year we have received several samples of moldy bread from various sources and so have been able to make a few more observations on the production of protoperithecia by wild-type races of *N. sitophila*. The "Beall" race, mating-type *A*, for example, produces very many large protoperithecia. The "Sands" race, also mating-type *A*, produces many definite protoperithecia, but they are small. A sample of moldy bread "HSS" was brought in by students of the Bronx High School of Science. Cultures from the bread produced some perithecia and so must have originally contained both mating-types. Many single tip cultures were all unisexual, mating-type *a*. Fifty single ascospore cultures were also studied. Thirty-five of these races were tested for mating-type. Eighteen were *A* and 17 were *a*. None of these races produced *easily recognizable* protoperithecia in any abundance on a malt extract agar medium. However, one could usually find scattered here and there quite a few small *colorless* bodies which could probably function as protoperithecia. It is such races as these that should be studied more extensively. We know from earlier studies that some races produce protoperithecia that continue to grow to reach the size of mature perithecia without fertilization. This HHS material represents races which do not develop characteristic protoperithecia, that is, receptive bodies easily seen with a hand lens, and which turn brown after a few days.

Professor P. D. Voth sent us several different cultures of *N. sitophila* taken from bread in Chicago. None of these produced what might be called "typical" protoperithecia. Voth 4, mating-type *a*, grown in plate cultures on cornmeal agar seemed to be entirely devoid of protoperithecia for a whole week, yet when conidiated with the Sands race this plate developed quite a few good perithecia and also numbers of good protoperithecia which were confined to the spot conidiated.

The writer, after some study of the wild-type races mentioned above, is not in a position to warrant any conclusions whatever on this matter of production of protoperithecia. Receptive bodies may often consist merely of differentiated special hyphal branches which would be called ascogonia. If they were not conidiated or spermatized they might not go on to grow into what we call protoperithecia. Whether or not production of ascogonia or protoperithecia is regulated by a single pair of factors is still a question. The results of the experiments with the non-conidial races 56.2 and 56.6 seemed to show rather definitely that factors *P* and *p* had been segregated according to Mendelian principles at reduction. No such clear cut segregation was evident when we studied such wild type races as the HHS and Voth races. Nevertheless, it is clear that in all studies of sterilities or incompatibilities which are concerned with the production of perithecia it is im-

portant to determine whether (and to what extent) the races being analyzed are capable of producing receptive bodies, be they simple ascogonia or the more complicated protoperithecia.

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SPORANGIAL PROPAGATION OF BLUE MOLD FUNGUS ON ASEPTICALLY GROWN TOBACCO SEEDLINGS

ROBERT A. STEINBERG

Peronospora tabacina Adam, which is the cause of the blue mold disease of tobacco, can be propagated only on its host. Its freedom from extraneous microorganisms cannot, therefore, be brought about by standard culture methods. The desirability of pure culture is apparent for nutrition studies with the fungus, and perhaps for checking symptoms obtained with this disease in nature.

It has been found possible to grow the fungus on one-month-old tobacco seedlings propagated aseptically in 200-cc. Erlenmyer flasks on 50 cc. of a mineral agar. The seedlings were grown at room temperature under 500 foot candles of intermittent or continuous light provided by 3500° white fluorescent lamps. Details of the procedure will appear elsewhere.[1] Seedlings of Maryland medium broadleaf and Xanthi Turkish proved satisfactory. Inoculation of seedlings was on the lower leaf surfaces. A straight sharpened needle was used to pick up a bit of filter paper pulp from sterile suspension, rubbed over a leaf covered with spores and placed on a healthy leaf. Almost invariably infection took place if the leaf tissue was injured and the mixture of spores and pulp applied at this point.

The flasks were then kept in the bottom of a lighted refrigerator[2] in a glass moist chamber at 45–50° F for 7 days. They were then removed from the moist chamber and placed in the top of the same refrigerator where the temperature was about 72° F. Spores began to appear on the upper surfaces of the inoculated leaves on the seventh day and increased in profusion up to the twelfth day. Viable spores were still present after infected plants were held in the lighted refrigerator for a total of 3 weeks and stored in the dark at 41° F for 2 additional weeks. Excellent propagation of the fungus was also possible at a uniform temperature of 59° F, obtained by a 25-watt tungsten lamp in a refrigerator of 5 cubic feet capacity.

The fungus was maintained in this manner for over a year, a total of 27 successive transfers. All contaminating microorganisms had disappeared by the third transfer. The symptoms of infection were similar to those in nature, except for greater profusion of spores on upper instead of lower leaf

[1] **Steinberg, Robert A.** Growth responses to organic compounds by tobacco seedlings in aseptic culture. Jour. Agr. Res.

[2] **Wolf, Frederick A. & McLean, Ruth A.** Sporangial proliferation in *Peronospora tabacina*. Phytopathology 30: 264–268. 1940.

surfaces. Other leaves of the plant than those inoculated often became infected. Blackening at the base of leaf midribs and of stems indicated the path of the fungus was, in some cases, through these tissues. Roots of infected plants remained apparently healthy.

Good sporulation was obtained on leaves detached immediately before inoculation, the bases of which had been plunged into the agar. Turgid seedlings growing on agar that had almost dried completely also permitted profuse sporulation of the fungus.

Light appeared necessary for sporulation, since no spores were formed if the flasks were wrapped in black paper during the latter 2 weeks of the 3-week period. Absence of light during the fungal vegetative period (first week after infection) retarded sporulation partially or completely, depending probably on the completeness with which light was excluded. Those seedlings in which sporulation was thus suppressed remained healthy subsequently. Growth of the seedlings after infection in a stream of CO_2-free air appeared to be without influence on development of the fungus. Growth of the fungus, therefore, would seem independent of the immediate products of photosynthesis. The fungus could not, however, be propagated sporangially on seedlings killed by vapors of carbon tetrachloride, ammonia, or alcohol.

Attempts to cultivate *P. tabacina* on media proved uniformly unsuccessful with many standard and experimental formulae. Materials aiding germination of spores always proved ineffective for growth in media. Germ tubes 10 or more times the spore diameter were often formed before growth stopped. The absence of all growth in these tests also served as a demonstration of the absence of contaminating microorganisms. Included in the different media tested were sap of the tobacco plant and various enzymes—all of which had been rendered aseptic through filtration.

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STRUCTURE AND REPRODUCTION OF TRICHOGLOEA REQUIENII, WITH A COMPARISON OF THE GENERA OF HELMINTHOCLADIACEAE*

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In spite of the fact that the Nemalionales are better known than any other order of the red algae as regards the developmental morphology of the genera, there are still, as in all large groups of organisms, a number of gaps to be filled before the outline of the picture is complete. Among the genera requiring study is the tropical genus *Trichogloea*. It belongs to the family Helminthocladiaceae, which includes among others the classical *Nemalion* as well as such familiar names as *Helminthocladia, Helminthora,* and *Liagora*. The material for the present study was collected in Hawaii during the years 1941 and 1942.

Butters (1903) has given a good account of the structure of the thallus and has described and illustrated certain stages in the development of the reproductive organs of one species. Nonetheless, the steps that lead up to the formation of the mature gonimoblast are not well enough understood to allow a detailed comparison with related genera.

As in *Liagora*, the thallus is lime-incrusted and the genus may at times be mistaken for the latter. This resemblance is purely superficial, however, for by the position and form of the carpogonial branch and by the development of the gonimoblast and associated structures, *Trichogloea* shows itself to be more closely related to *Nemalion* than to any of the other genera of the Helminthocladiaceae which have been investigated. Before taking up the structure and reproduction, it may be well to give a brief summary of the history and geographical distribution of the genus and to discuss the reasons for identifying the plants studied with *T. Requienii* (Mont.) Kütz.

The genus *Trichogloea* was established by Kützing in 1847 upon a plant from the Red Sea which Montagne (1843) had described a few years earlier as *Batrachospermum Requienii*. Since that time, two additional species have become known: *T. lubrica* J. Agardh (1876) and *T. subnuda* Howe (1934). For a complete record of the early literature on the genus, reference should be made to the "Sylloge Algarum" of De Toni (1897).

After the description by Montagne, *T. Requienii* was reported from the Red Sea for a second time by Zanardini (1858), who also gave the first good illustration of the habit of this alga. Later, the species was recorded from

* The publication of the illustrations has been assisted by the Lucien M. Underwood Memorial Fund.

Madagascar (Pilger 1908), Mauritius (Børgesen 1942), Ceylon and the Malayan Archipelago (Weber-van Bosse 1921); and to judge from the descriptions and illustrations of Okamura (1923, 1930, 1936), it seems very likely that the plants from Japan which he has referred to *T. lubrica* are also representative of *T. Requienii*. The species is here reported for the first time from Hawaii.

Trichogloea lubrica was described by J. Agardh in 1876 from a specimen of the plant which Harvey had distributed as no. 46 of his "Friendly Islands Algae" under the manuscript name *Liagora lubrica*. Subsequently the species was reported from various places other than the Tonga Islands. None of these records, however, seems to be based upon true *T. lubrica*. The reports from Mauritius by Jadin (1934) and others have been shown by Børgesen (1942) to apply to *T. Requienii*, those from Hawaii by Tilden (1901b) and Butters (1903) have been found by Howe (1934) to be based upon *T. subnuda*, and in the paragraph above the opinion is expressed that the reports from Japan apply to *T. Requienii*. The only other records of *T. lubrica* are those from Guadeloupe (cf. De Toni 1897) and Barbados (Vickers 1905) in the West Indies. These reports are probably based upon an undescribed species, which may be identical with the plant from Bermuda (Collins and Hervey 1917; Howe 1918) which passes under the manuscript name of *T. Herveyi* Setchell.

The third species of *Trichogloea*, *T. subnuda*, was described by Howe (1934) from material from Hawaii. In the same paper, he also pointed out that the Hawaiian specimens which Tilden (1901a) had distributed as *Nemalion ramulosum* Harv. under no. 419 of her "American Algae, Century V" actually were *T. subnuda*. This material was also published by Tilden (1901b) under the name *T. lubrica* and part of it formed the subject of a paper by Butters (1903) entitled "Observations on *Trichogloea lubrica*." Thus far *T. subnuda* is known from only two localities on the island of Oahu: Kahuku Point, where it was obtained among the driftweed by Miss Tilden in June, 1900; and Kaneone Bay, where it was secured by Galtsoff in July, 1930. Both these stations are situated on the northeastern side of the island. Most of the writer's specimens of *T. Requienii* were collected along the southern shores of Oahu.

As will be seen from the photographic reproductions which Howe (1934) has given either of the type or of authentic specimens of the three validly published species of *Trichogloea*, *T. lubrica* is a much more robust plant than either *T. Requienii* or *T. subnuda*. The plump habit of *T. lubrica* is also shown by a duplicate of the original material of Harvey (Friendly Islands Algae, no. 46) which is in Herb. Univ. Calif. (no. 696847) as well as by a photograph in this Herbarium of the Tonga Islands specimen of Harvey which is in Herb. Sydney Bot. Garden.

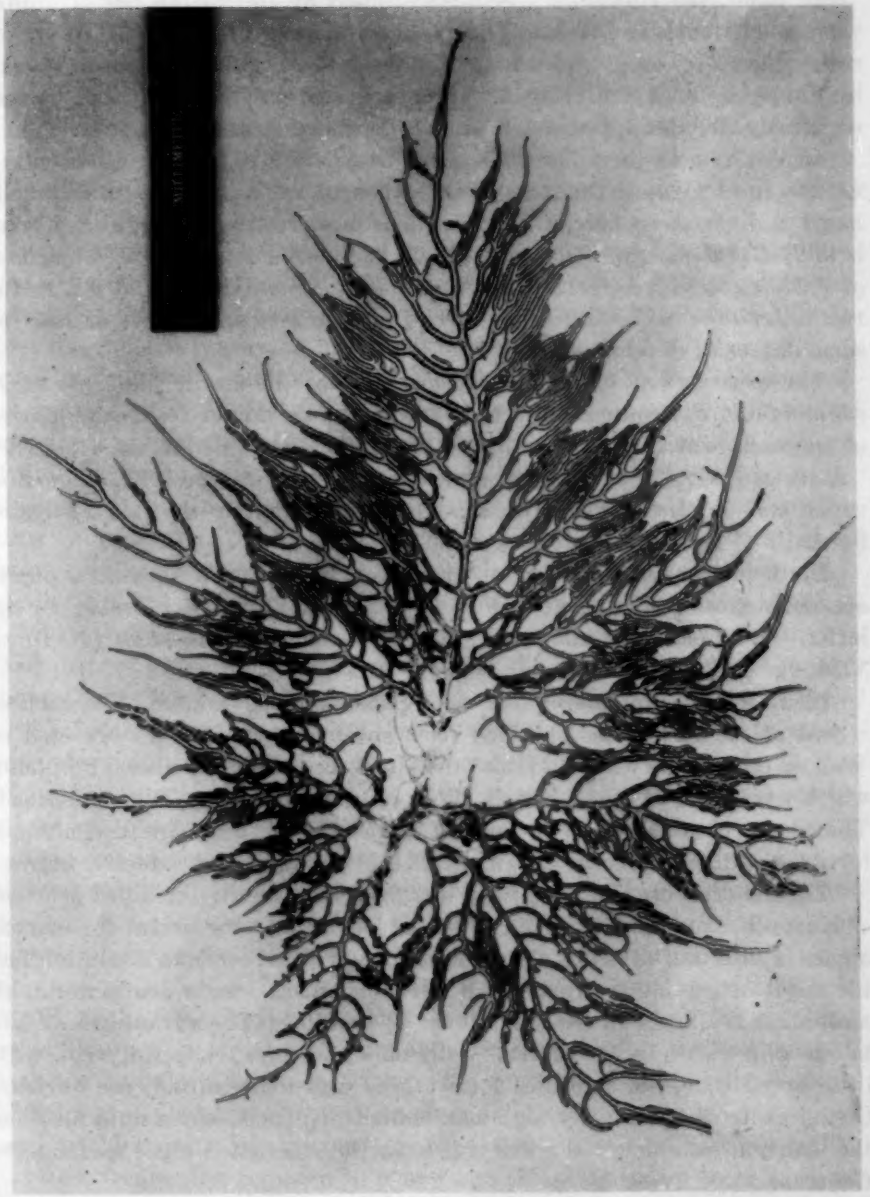


FIG. 1. *Trichogloea Requienii*. Habit of plant from Waikiki, Oahu, Hawaiian Islands (Herb. Univ. Calif. no. 696848). Leg. G. F. Papenfuss, March 21, 1941.

In habit, the Hawaiian specimens studied by the writer (fig. 1) differ very much from *T. lubrica*. The plants are identified with *T. Requienii* rather than *T. subnuda*, which, as has been said, was described from Hawaii by Howe, because the cells of the carpogonial branch give rise to comparatively well developed sterile or so-called involucral filaments.

In *T. subnuda*, these filaments, according to Howe, are of a rudimentary nature. In fact, this is the structural feature upon which the specific name is based and the one whereby *T. subnuda* is most readily distinguished from both *T. Requienii* and *T. lubrica*. Howe established this from a comparison of *T. subnuda* with authentic material of *T. Requienii* and *T. lubrica*. In the two latter species, he found the sterile filaments to show more or less the same degree of development.

The observations of Howe on the nature of the sterile filaments of *T. subnuda* and *T. Requienii* have been verified by the writer from examination of an example of *T. subnuda* which Miss Tilden distributed as no. 419 of her "American Algae," and of a fragment of the female type of *T. Requienii*, which was obtained from Museum Hist. Nat. Paris by the late Professor Setchell.

In their irregular manner of branching the writer's Hawaiian plants also show greater agreement with *T. Requienii* than with *T. subnuda*. In the latter, the branches are more or less dichotomous in arrangement (cf. Howe 1934, fig. 1).

In Hawaii, *T. Requienii* is a spring and summer annual. The earliest record of the season was obtained from specimens which were collected at Waikiki on February 24, 1941, and the latest record was furnished by plants which were secured on August 23, 1941, on the shore below Diamond Head. The plants obtained on February 24 were up to 7 cm. high and already fertile; and those collected on August 23 still contained reproductive organs.

The material used in this study was fixed in a slightly modified solution of Karpechenko.[1] This fixative the writer has found very useful for marine algae. In addition to being a good fixing agent, Karpechenko's solution has the merit of leaving no apparent deleterious effects even when material of most algae is allowed to remain in it for several days. The advantages of this to one who wishes to fix material while on a few days' collecting trip, with little or no time, nor facilities for washing and dehydrating, are obvious. Owing to its comparatively high acid content, Karpechenko's fluid also has the utility of completely decalcifying such lime-incrusted algae as *Liagora*, *Galaxaura*, and *Trichogloea*.

Selected parts of the plants were stained in 1 per cent anilin blue ac-

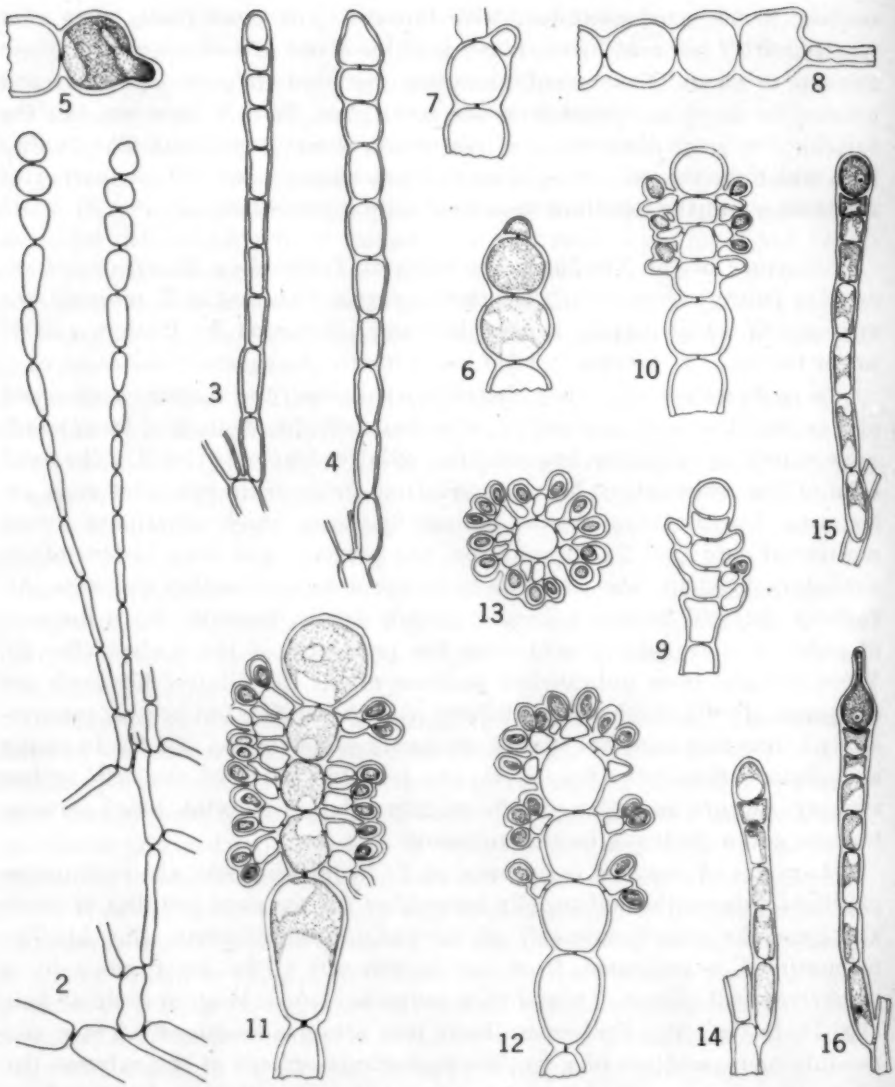
[1] This solution was made up as follows: Part A—chromic acid 1 gm., glacial acetic acid 5 cc., sea water 65 cc. Part B—commercial formalin 40 cc., sea water 35 cc. Parts A and B are kept separately and equal amounts are mixed as required.

cording to the usual procedure with this dye. The small pieces were next treated with 1 per cent hydrochloric acid for about fifteen minutes, washed, run up to about 70 per cent glycerine, mounted in pure glycerine, and crushed by applying pressure to the cover-glass. With a little practice the suitable degree of dissociation of the tissues is easily obtained. The facility with which the tissues will separate depends largely upon the consistency of the thallus and the length of treatment with hydrochloric acid.

Structure of the Thallus. The habit of *Trichogloea Requienii* is indicated in figure 1. Structurally the thallus agrees with that of *T. subnuda*, the anatomy of which has been described and illustrated by Butters (1903) under the name *T. lubrica*. A brief account will consequently suffice here.

As in *Nemalion* and other Helminthocladiaceae, the thallus is composed of a central tissue of intertwined, branched, colorless filaments, from which issue numerous radiating, branched, assimilative filaments (fig. 2). The basal cells of the assimilatory branches send out from their proximal ends descending, but at times also ascending, filaments which contribute to the multiaxial core (fig. 2). In addition, the primary and later orders of assimilatory filaments also give rise to higher orders of similar filaments. All radially directed filaments usually remain simple, however, for a distance of eight or more cells inward from the periphery of the thallus (fig. 2). When mature, these unbranched portions of the assimilative filaments are composed of cells which are moniliform near the surface but become progressively longer and narrower toward the center of the thallus (fig. 2). In young assimilatory filaments (figs. 3, 4), the bead-like form of the cells is less striking. Mature assimilative cells contain a large, parietal, lobed chromatophore and a centrally located pyrenoid.

Hairs are of common occurrence in *T. Requienii*. They are initiated as papilloid outgrowths and usually issue from the terminal cell, but at times also from the penultimate cell, of the assimilative filaments (fig. 5). The primordium is separated from the mother-cell at an early stage by a transverse wall (figs. 6, 7), and then grows out into a long, unicellular hair (fig. 17, to the left). Full-grown hairs may attain a length of 0.9 mm. and possibly more, and are of a hyaline appearance, except at the extreme tip, where the cytoplasm is dense. At any one time, a cell bears but one hair. The hairs are ephemeral and may be more or less completely lacking in old plants or older parts. In vigorously growing parts, a cast-off hair is usually, or at least frequently, replaced by a new one (fig. 7). Several crops of hairs may thus be produced by a cell. Not infrequently a new hair may be in the course of formation while the old one is still intact (fig. 17, to the left). Often the new hair is initiated at the place of attachment of the preceding one and can be seen projecting into its empty base. An old hair is shed as



FIGS. 2–16. *Trichogloea Requienii.* FIG. 2. Part of dissociated thallus showing a few of the longitudinal axial filaments (at the lower end) and two of the radiating, assimilative filaments. ×300. FIGS. 3, 4. Young assimilative filaments. ×600. FIGS. 5–7. Early stages in the formation of hairs from the terminal cells of assimilative filaments, figure 7 also showing the empty base of a shed hair. ×600. FIG. 8. Distal end of an assimilative filament and the basal part of an old hair, which shows an early stage in the formation of the constriction which brings about the abscission of hairs. ×600. FIGS. 9–13. Stages in the development of antheridia, figures 9–12 being optical median longitudinal sections of the terminal ends of assimilative filaments and figure 13 an optical transverse section of an assimilative cell. ×775. Figure 9 shows cells which are in an early stage of cutting off antheridial mother cells, one of which has formed an antheridium. Figures 10–13 represent older stages, mature antheridia being shown in figures 11–13. FIGS. 14–16. Early stages in the development of carpogonial branch. ×600. In figure 14 the young carpogonium still contains a chromatophore, figure 15 shows a stage at which the chromatophore has broken down, and in figure 16 the trichogyne is just being formed.

the result of a constriction which develops immediately above its place of insertion (fig. 8). For a summary of knowledge concerning the hairs of Florideae and their probable function in the absorption of nitrates reference should be made to the accounts of Rosenvinge (1911) and Kylin (1937, pp. 37–38).

Development of Antheridia. Although *Trichogloea Requienii* is ordinarily dioecious, occasional plants are monoecious. *T. subnuda* on the other hand, is monoecious according to both Butters (1903) and Howe (1934).

The antheridia are formed on the distal cells of the assimilatory filaments (figs. 9–12). From one to five successive cells may be concerned in their production. Usually the terminal cell remains sterile (figs. 10, 11) but in rare instances it also becomes fertile (fig. 12). Not infrequently the subterminal cell, as well as the terminal cell, or at times even the one below it, remains sterile. In *T. subnuda*, on the other hand, to judge from the account and figures of Butters, the terminal cell of a fertile filament usually forms antheridia.

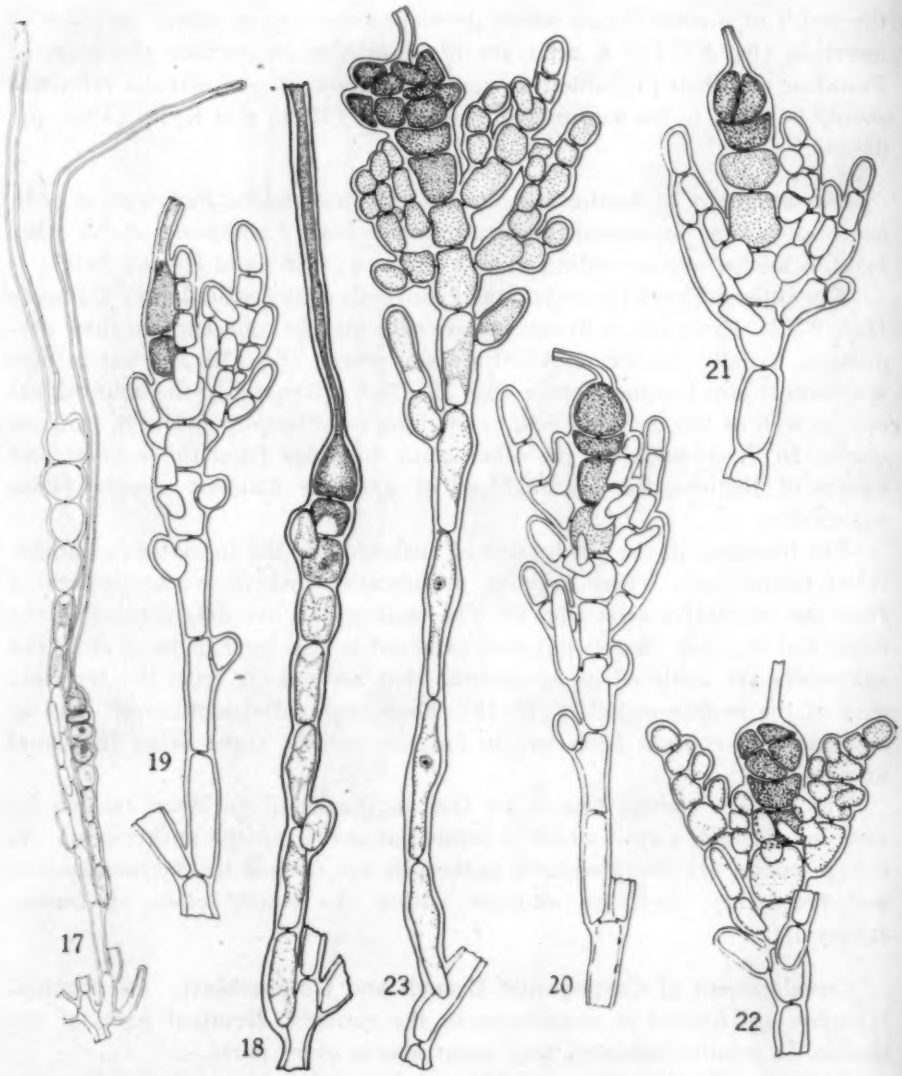
The first step in the production of antheridia is the formation of antheridial mother-cells. These arise as proliferations which become separated from the vegetative cells (fig. 9). The mother-cells are first formed at the distal end of a cell (fig. 9) but soon afterwards also from its basal end. The antheridia are initiated as outgrowths that are cut off from the terminal ends of the mother-cells (fig. 10–13). Each antheridial mother-cell may at any one time contain from two to four antheridia, three being the usual number.

The mature spermatium is set free in the usual floridean fashion by escaping through a split which is formed at the tip of the antheridium. As the spermatia are liberated, new antheridia are formed by the mother-cells and frequently they are situated within the empty cases of former antheridia.

Development of Carpogonial Branch and Gonimoblast. Carpogonial branches are formed in abundance in the younger, terminal parts of the thallus. In smaller numbers, they occur also in older parts.

In *Trichogloea* the carpogonial branch is a modified assimilative filament. This is the case in *Nemalion* also (cf. Kylin 1930, p. 8; 1937, p. 197) but is in contrast to that in the other genera of Helminthocladiaceae. In these the carpogonial branch is formed as an accessory branch by a cell of the radial filaments of the thallus, and is a specialized organ from the time of its inception.

According to Cleland (1919), the carpogonial branch of *Nemalion* can be recognized even in the one-celled stage. This, however, is not the case in *Trichogloea*. Here the future carpogonial branch is morphologically indis-



FIGS. 17–23. *Trichogloea Requienii*. FIG. 17. Almost mature carpogonial branch (to the right) and its supporting assimilative filament, whose terminal cell bears a mature hair and is in course of initiating a new hair. ×315. FIG. 18. Mature carpogonial branch with the cytoplasm of the hypogynous cell somewhat denser than that of the cells proximal to it and two of the cells bearing young sterile filaments. ×750. FIG. 19. Carpogonial branch shortly after fertilization of carpogonium. ×750. The cytoplasm of the carpogonium is separating from that of the trichogyne, the chromatophore of the hypogynous cell is breaking down and the cytoplasm has become dense, and sterile filaments are in an active state of formation and growth. The chromatophores of the cells of the carpogonial branch proximal to the hypogynous cell and of the cells of the sterile filaments are not indicated. FIGS. 20–23. Early stages in the development of the gonimoblast. ×750. FIG. 20. Carpo-

tinguishable from a young assimilative filament. Staining also fails to bring out differences. The carpogonial branch first becomes recognizable at the time that the carpogonium is being differentiated. In such stages the terminal cell, or young carpogonium, of a fertile filament (fig. 14) is frequently a little larger than the apical cell of a young assimilative filament of comparable age (fig. 3). At this time, the young carpogonium still contains a chromatophore. Somewhat later (fig. 15), the carpogonium undergoes further enlargement, its chromatophore starts to break down and its cytoplasm begins to assume a homogeneous, dense, darkly-staining quality. Consequently such stages may easily be distinguished from young vegetative filaments.

Figure 16 shows the trichogyne of the carpogonium in an early stage. It will be seen that this carpogonial branch, like those shown in figures 15, 17, and 18, is composed of eight cells. The number of cells varies between five and ten; usually it is seven or eight. When fully developed, the trichogyne is about three times as long as the remainder of the carpogonial branch and projects well beyond the surface of the thallus (fig. 17). The cytoplasm of the trichogyne is homogeneous and faint except at the extreme tip, where it is fairly dense (fig. 17).

Before fertilization, the cells of the mature carpogonial branch proximal to the hypogynous cell begin to initiate sterile filaments (fig. 18). Additional sterile filaments are formed after fertilization has occurred, as will be discussed later.

At the stage indicated in figure 18, the cytoplasm of the hypogynous cell also begins to assume a dense, darkly-staining quality. Such a change has also been reported by Butters as occurring in this cell of the mature carpogonial branch of *T. subnuda*.

Noticeable signs that fertilization has taken place are the separation of the cytoplasm of the carpogonium from that of the trichogyne, a withering of the trichogyne, a breaking down of the chromatophore of the hypogynous cell and a further increase in density of its cytoplasm, and an accelerated production of sterile filaments (fig. 19).

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gonial branch whose fertilized carpogonium has become divided transversely into a small stalk cell and a larger (distal) primary gonimoblast cell. The chromatophore of the cell below the hypogynous cell has degenerated. Those of the other cells of the carpogonial branch and of the cells of the sterile filaments are not indicated. FIG. 21. Part of a carpogonial branch, showing the first division of the primary gonimoblast cell. The chromatophores of the cells of the sterile filaments and of the cells of the carpogonial branch proximal to the cell below the hypogynous cell are not indicated. FIG. 22. A young gonimoblast and part of the carpogonial branch and its sterile filaments. The unstippled cells contained chromatophores while those of the other cells had broken down and the cytoplasm had taken on a dense, homogeneous appearance. FIG. 23. Optical longitudinal section of a carpogonial branch and a gonimoblast which is slightly farther advanced than the one shown in figure 22. The five lower cells of the carpogonial branch and the cells of the sterile filaments issuing from some of them still contained chromatophores.

The gonimoblast-producing function of the fecundated carpogonium next becomes evident. In *T. Requienii* the gonimoblast is initiated by a transverse division of the carpogonium. The gonimoblast develops from the distal of the two cells so formed. This primary gonimoblast cell is always larger than the lower so-called stalk cell (fig. 20).

The stalk cell undergoes no further division, while the primary gonimoblast cell by division gives rise to the gonimoblast. The first division is always by a wall which is oblique with reference to the preceding cleavage plane (fig. 21). According to Butters, the first division of the primary gonimoblast cell of *T. subnuda* is by a tranvserse wall. His figures illustrating this are not entirely convincing but if this is so, we have another feature whereby *T. subnuda* could be distinguished from *T. Requienii*.

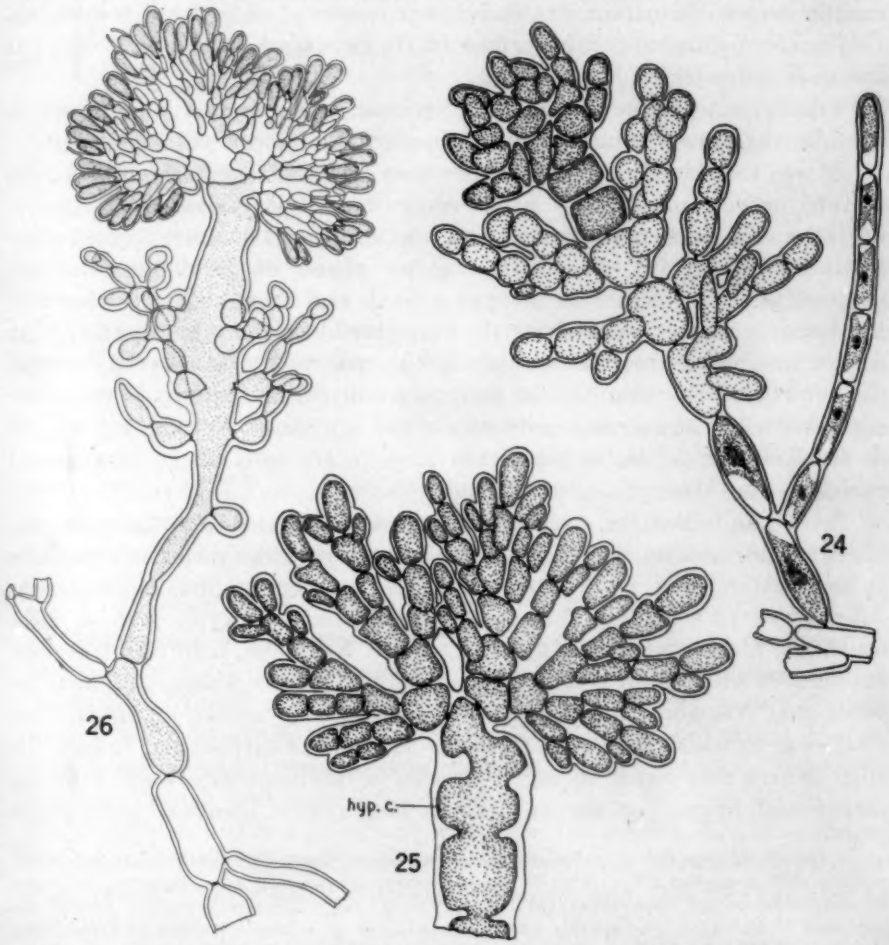
The further development of the gonimoblast is illustrated in figures 22–26. It will be seen that the derivatives of the primary gonimoblast cell form a globular aggregation of cells (figs. 22, 23), which serve as the starting points of radiating, branched gonimoblast filaments (fig. 24). The terminal cells of these are transformed into carposporangia (figs. 25, 26).

Concurrently with the growth and development of the gonimoblast, a number of striking changes take place in the cells of the carpogonial branch and of the sterile filaments.

Starting at the distal end, there is a progressive degeneration of the chromatophore of the cells of the carpogonial branch and an increase in density of their cytoplasmic content. As has been pointed out, one of the early signs that fertilization has occurred is the breaking down of the chromatophore of the hypogynous cell and an accompanying increase in density of its cytoplasm (fig. 19). In the stages shown in figures 20 and 21, this secondary effect of fertilization had advanced to the cell below the hypogynous cell. In those depicted in figures 22 and 23, it had progressed to the second cell and in that shown in figure 24 to the third cell proximal to the hypogynous cell. Ultimately, when the gonimoblast approaches maturity, this change is observed to have taken place in all the cells of the carpogonial branch; and at times even the supporting cell will have been affected (fig. 26).

During the later phases of maturation of the gonimoblast, the cells of the carpogonial branch undergo another conspicuous change. There is first a gradual widening of the pit-connections between them. This change, like the one described above, takes place in a progressive sequence, starting at the basal cell of the gonimoblast and extending downward to the lowermost cell of the carpogonial branch. Thus, in advanced stages, it is found that the originally narrow pit-connections have become wide canals (fig. 25). In such stages, it is still possible to recognize the limits of the individual cells. But later, when the gonimoblast is full-grown, a complete coalescence

of the cells will result in the establishment of a large fusion or placental cell (fig. 26). In addition to the cells of the carpogonial branch, this cell incorporates the stalk cell, the basal cells of the gonimoblast and a few of the cells of the sterile filaments. At times the lowermost cell or two of the



FIGS. 24–26. *Trichogloea Requienii*. FIG. 24. Optical longitudinal section of a carpogonial branch and a young gonimoblast. ×750. In the solidly stippled cells the chromatophore had degenerated. FIG. 25. Almost mature gonimoblast with carposporangia in an early stage of formation. ×750. FIG. 26. Mature gonimoblast and its placental cell. ×360. hyp. c., hypogynous cell.

carpogonial branch do not participate in the formation of the placental cell.

It is of interest that a similar fusion-cell is neither mentioned nor illustrated by Butters as occurring in *T. subnuda*, and to judge from his figure 16, which is of a mature gonimoblast, it seems reasonably safe to

conclude that actually a placental cell is not formed in this species. If this is so, the absence of a fusion-cell in *T. subnuda* would not only be an additional but a very clear-cut feature distinguishing it from *T. Requienii*. The likelihood of this difference receives indirect support from the fact that exactly such a distinction obtains between species of the related *Helminthocladia*. Here a fusion-cell is formed in *H. Papenfussii* (Martin 1939) but not in *H. calvadosii*[2] (Kylin 1930).

Finally, with respect to the post-fertilization changes, it is necessary to consider the growth, function and ultimate fate of the sterile filaments.

It was mentioned earlier that, previous to fertilization, the cells of the mature carpogonial branch proximal to the hypogynous cell begin to initiate sterile filaments (fig. 18)[3] and that additional ones are formed after fertilization (fig. 19). During the earlier phases of development of the gonimoblast, these filaments through growth and branching give rise to a loose aggregation of cells about the carpogonial branch (figs. 20–24).[4] At first the cells are provided with a chromatophore, but in correlation with the growth of the gonimoblast there occur a disintegration of the chromatophores and an increase in density of the cytoplasm (figs. 22–24). It will be recalled that similar changes take place in the cells of the carpogonial branch during these phases of development.

According to Butters, sterile filaments are not formed in *T. subnuda* previous to fertilization. However, this point requires substantiation inasmuch as he (Butters 1903, p. 18) states that "No entirely mature and unfecundated procarps were seen." In *Nemalion multifidum* (Kylin 1916, p. 265) the sterile filaments begin to appear, as in *T. Requienii*, before fertilization has been effected. With respect to the position of these filaments, it may be noted that *Nemalion* and *Trichogloea* are the only genera of Helminthocladiaceae in which they arise from the cells of the carpogonial branch. In other genera they either spring from cells in the immediate vicinity of the carpogonial branch or are entirely lacking. Their identical position in

[2] The specific epithet of *Helminthocladia calvadosii* (Lamour.) Setchell is customarily written with a capital "C." This is not necessary, because the name-bringing synonym *Dumontia calvadosii* Lamouroux (in Duby 1830, p. 941) commemorates the French department of Calvados. The species was first mentioned as a *nomen nudum* by Lamouroux in volume 5 (1824) of "Dict. Class. Hist. Nat." (Not "Dict. Hist. Nat.," as sometimes cited.)

[3] Pilger (1908, fig. 1) illustrates a carpogonial branch of *T. Requienii* in which the hypogynous cell bears a sterile filament. Since his figures are rather diagrammatic, one may question the accuracy of this delineation. In *T. subnuda* they are not formed by the hypogynous cell (Butters 1903).

[4] Schmitz (1896, fig. 203c) has illustrated the sterile filaments of *T. Requienii* as extending well beyond the gonimoblast. However, his figure is so reminiscent of the condition in *Helminthora divaricata*, as illustrated by Thuret and Bornet (1878, pl. 32, figs. 1, 9, 10), that it probably is representative of this species. Neither Pilger (1908) nor Børgesen (1942) found the filaments to extend beyond the base of the gonimoblast.

Nemalion and *Trichogloea* is further evidence in favor of the belief that these two genera are more closely related to each other than to other genera of Helminthocladiaceae.

The sterile filaments of Helminthocladiaceae have usually been regarded as performing a kind of protective role and have frequently been designated as involueral filaments. Although this may conceivably be their function in certain species of *Helminthocladia* and *Liagora* such is not the case in *Trichogloea*. Here, as in *Nemalion* and certain other Nemalionales (cf. Kylin 1937), the sterile filaments constitute a nurse tissue which supplies the gonimoblast with nutrient materials. This is particularly clear from a study of advanced stages of the gonimoblast. In such stages (fig. 26) it is observed that the originally narrow pit-connections of the sterile cells have become wide canals, the cells have lost their dense content, and the walls have undergone gelatinization. Old stages give the impression, moreover, of having suffered a loss of sterile cells (compare figs. 24 and 26), which suggests a possible disintegration of some of the cells after the complete absorption of their content by the placental cell and gonimoblast.

As has been mentioned, a few of the lowermost sterile cells become incorporated in the placental cell. If the carpogonium is not fertilized, the sterile filaments of *T. Requienii* develop into ordinary assimilative filaments.

Monosporangia, such as has been described by Svedelius (1917) for *Helminthora divaricata* and by Abbott (1945) for *Liagora farinosa* were not observed.

Comparison of the Genera of Helminthocladiaceae. In the foregoing pages comparison was repeatedly made between *Trichogloea* and its closest ally, *Nemalion*. Attention was also drawn to certain points of agreement with or departure from other related genera. It now seems desirable to make a somewhat comprehensive comparison of all genera of Helminthocladiaceae which have been investigated.

In addition to *Trichogloea* and *Nemalion*, this family, as now delimited, includes the following eight genera: *Helminthocladia*, *Helminthora*, *Liagora*, *Dermonema*, *Cumagloia*, *Ardissonea*, *Endosira*, and *Dorella*. The last five are monotypic.[5]

Detailed developmental studies have been made of representatives of seven of the genera. Nothing or at best very little is known concerning the development of *Ardissonea*, *Endosira*, and *Dorella*,[6] and they consequently have to be left out of the following discussion.

[5] The genus *Dermonema* used to be credited with two species, *D. dichotomum* and *D. gracile*, but Weber-van Bosse (1921) has shown that these binomials apply to the same plant, whose name should be *D. gracile* (Mert.) Schm. However, it was recently established by Børgesen (1942) that the correct name was *D. Frappieri* (Mont. & Millard) Børg.

[6] *Dorella* was described by Weber-van Bosse (1921) from a plant from the East Indies, which she distinguished from *Helminthocladia* by its lack of sterile filaments about

In agreement with the majority of Nemalionales, the family Helminthocladiaceae is composed of forms that are haplobiontic. Although differing in detail the genera of this family also show close anatomical correspondence. In all of them the thallus is multiaxial and the assimilative tissue is in the form of radially disposed, branched filaments which issue from the central strands. In *Liagora* and *Trichogloea* the thallus is lime-incrusted.

As to the male reproductive organs, there are differences in the position and arrangement of the antheridia and their mother-cells on the assimilative filaments, but here again a good deal of agreement obtains among the genera.

Significant differences exist, however, with respect to the position and nature of the carpogonial branch and the development of the gonimoblast and associated structures.

As has been pointed out, the carpogonial branches of *Nemalion* and *Trichogloea* are modified assimilative filaments. In the other genera, with the possible exception of certain species of *Liagora,* they are formed as specialized, accessory filaments by cells of the assimilative tissue. (In *Dermonema* they occur on the inner, colorless cells of the assimilative tissue system; Svedelius 1939.) To judge from the illustrations of Yamada (1938, pp. 30–34) it seems likely that in the section *Mucosae* of *Liagora* the carpogonial branch is a modified assimilative filament.

With respect to the early development of the gonimoblast, it is to be noted that this structure develops from the distal of the two cells formed in consequence of the first division of the fertilized carpogonium in *Trichogloea, Nemalion* (Kylin 1916), *Helminthora* (Thuret and Bornet 1878; Kurssanow 1909; Kylin 1928) and *Liagora* (Kylin 1930), while in *Helminthocladia* (Kylin 1930; Martin 1939) both the first-formed cells participate in the production of the gonimoblast. In *Dermonema* (Svedelius 1939) and *Cumagloia* (Gardner 1917; Kylin 1928; Smith 1938) gonimoblast filaments issue directly from the sides of the carpogonium. No transverse division of the carpogonium occurs here. (In *Dermonema* the fertilized carpogonium first establishes a connection with the supporting cell.) The mature gonimoblast is a comparatively compact, globose aggregation of cells arranged in filaments in all genera excepting *Dermonema* and *Cumagloia,* in which it is a diffuse, filamentous structure.

The formation or non-formation of a placental cell is an unstable feature which in certain instances even varies in species of the same genus. Thus a fusion-cell is formed in *Trichogloea Requienii* (fig. 26) but apparently not

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the gonimoblast, its small stature, and its unbranched thallus. Inasmuch as Kylin (1930) has shown that one of the European species of *Helminthocladia* lacks sterile filaments and since size and the absence or presence of branching are characters of questionable generic value, it becomes doubtful if *Dorella* should be retained.

in *T. subnuda* (Butters 1903, fig. 16). In *Helminthocladia Papenfussii*, Martin (1939, figs. 20, 21) observed a well-developed placental cell while such a cell, according to Kylin (1930), is not formed in *H. calvadosii*. Although a placental cell is formed in *Nemalion multifidum* (Kylin 1916) this, to judge from the figures of Børgesen (1915–1916), is not the case in all species of this genus. In *Liagora viscida*, Kylin (1930) observed the formation of a fusion-cell, but the figures of Børgesen (1915–1916) and Yamada (1938) suggest that such a cell is not formed in certain other species of *Liagora*. Only one species of *Helminthora*, *H. divaricata*, has been thoroughly studied and in it a placental cell is not formed (Kylin 1928). According to Smith (1938), a partial coalescence of the cells of the carpogonial branch occurs in *Cumagloia* and from the work of Svedelius (1939) it may be concluded that this is the case in *Dermonema* also.

In regard to the presence or absence of sterile filaments about the carpogonial branch or gonimoblast, there again is a good deal of variation among the genera and in some instances even among species of the same genus. In *Trichogloea* sterile filaments are present. They arise from the carpogonial branch and begin to be formed at least in *T. Requienii* before fertilization has taken place. The same is true of *Nemalion* (Kylin 1916). In these genera, the filaments have no protective value but function as a nurse tissue to the gonimoblast. In *Helminthora* sterile filaments are also formed. They are initiated after fertilization has occurred, and spring not from the cells of the carpogonial branch but from the two vegetative cells immediately above the supporting cell (Kylin 1928). According to Fritsch (1945, p. 614) the filaments arise from the second and third cells of the carpogonial branch. This, however, is a misinterpretation of the statement and figures of Kylin. The sterile filaments of *Helminthora* perform a protective as well as a nutritive function (Kylin, *op. cit.*).

In *Helminthocladia* sterile filaments of a protective kind are present in the South African *H. Papenfussii* (Martin 1939). They are formed after fertilization has taken place and arise from the vegetative cell immediately above the supporting cell. In the European *H. calvadosii*, on the other hand, sterile filaments are not formed according to Kylin (1930). The observations of Kylin are at variance with those of Schmitz (1896) and Rosenvinge (1909), who also worked with European material. Schmitz does not specify the species upon which his observations were made, but Rosenvinge studied *H. purpurea*, which is regarded as synonymous with *H. calvadosii*.[7] Martin (*op. cit.*) has suggested that Kylin, who studied material from the Atlantic coast of France, may have had a species which was different from that studied by Schmitz and Rosenvinge. (Rosenvinge's material came from Den-

[7] Rosenvinge describes the sterile filaments as issuing both from cells of the carpogonial branch and cells of the supporting filament. Their origin from the carpogonial branch is in need of substantiation.

mark.) This seems very likely, especially since *Helminthocladia* is represented in Europe by a second species, *H. Hudsoni;* and according to Hamel (1930) these species at times closely resemble each other.

Sterile filaments do not occur in *Liagora viscida* (Kylin 1930), but filaments of a protective kind are formed from the vegetative cell (or cells ?) below the supporting cell in a variety of species of *Liagora* (cf. Børgesen 1915–1916; Yamada 1938). *Cumagloia* entirely lacks sterile filaments (Kylin 1928), and they apparently are not formed in *Dermonema* either (Svedelius 1939).

On the basis of its diffuse gonimoblast, Schmitz in 1896 erected for *Dermonema* the subfamily Dermonemeae. If this subfamily is to be retained, it is clear from the investigations of Gardner (1917), Kylin (1928), and Smith (1938) that *Cumagloia* also is to be assigned to it.

From his recent study of *Dermonema*, Svedelius (1939) is inclined toward elevation of the Dermonemeae to the status of family. However, as is apparent from the preceding comparisons, *Dermonema* and *Cumagloia* show so many features of correspondence with other Helminthocladiaceae, that there seems to be little justification for separation even as to subfamily.

In these two genera, the gonimoblast is formed by the entire carpogonium instead of as in the majority of other genera by the distal of the two cells resulting from its first division. However, in *Helminthocladia* the entire carpogonium also operates in the production of the gonimoblast, even though in some species it first divides by a transverse wall.

In *Helminthocladia*, as in the majority of Helminthocladiaceae, the mature gonimoblast is a compact mass of cells. In *Dermonema* and *Cumagloia*, on the other hand, it is a diffuse structure. Inasmuch as the gonimoblast of these three genera is initiated in much the same way, that is, from the entire carpogonium, it would seem that the diffuse condition in *Dermonema* and *Cumagloia* as contrasted with the compact state in *Helminthocladia* is to be looked upon as primarily a difference of form.

The condition in *Dermonema* and *Cumagloia* has parallels, moreover, in the related family Batrachospermaceae. Here a diffuse gonimoblast occurs in *Sirodotia* (Kylin 1912; Skuja 1931a) and in *Nothocladus* (Skuja 1934), and what is even more significant, in at least one species of *Batrachospermum*, *B. orthostichum* (Skuja 1931b), a genus which is otherwise characterized by a compact gonimoblast.

The evidence at hand thus seems to favor retention of *Dermonema* and *Cumagloia* within the family Helminthocladiaceae.

SUMMARY

Although the order Nemalionales is better known than any other order of red algae in regard to the development of the gonimoblast, a number of

outstanding gaps remain. One of the genera of the family Helminthocladiaceae of which but little is known is the tropical *Trichogloea*.

The material for the study of this genus was secured in Hawaii. The reasons for identifying the plants with *T. Requienii* are discussed and an outline is given of the history of the genus and of the geographical distribution of its three species.

As in *Liagora*, the thallus is lime-incrusted. Structurally it agrees with other Helminthocladiaceae. That is, the thallus is composed of a central tissue of intertwined, branched, colorless filaments, from which issue radiating, branched, assimilative filaments. Hairs grow out from the terminal and rarely the subterminal cells of the assimilative filaments.

Ordinarily, *T. Requienii* is dioecious, but occasional plants are monoecious.

The antheridia are formed on the distal, subterminal cells of the assimilative filaments.

The majority of carpogonial branches are formed in the younger parts of the thallus. As in *Nemalion*, the carpogonial branch is a modified assimilative filament. Before fertilization, the cells of the carpogonial branch proximal to the hypogynous cell begin to form sterile filaments, the chromatophore of the carpogonium degenerates, and its cytoplasm becomes homogeneous and dense.

After fertilization, the carpogonium divides by a transverse wall. The proximal of the two cells so formed, the stalk cell, undergoes no further division while the distal cell, the primary gonimoblast cell, by division gives rise to the gonimoblast. When mature, the gonimoblast consists of a globose, comparatively compact, aggregation of cells arranged in filaments. The terminal cells of these become transformed into carposporangia.

During the early phases of development of the gonimoblast, there is an accelerated production of sterile filaments by the carpogonial branch. These filaments function as a nurse tissue to the gonimoblast.

Concurrently with the development of the gonimoblast there also occurs a degeneration of the chromatophore of the cells of the carpogonial branch and an accompanying increase in density of the cytoplasm. In later stages there is a widening of the pit-connections between these cells, which results, ultimately, in a complete coalescence of the cells and the establishment of a large placental cell.

Finaly, a comparison is made of the genera of the Helminthocladiaceae with particular reference to the position and nature of the carpogonial branch and the development of the gonimoblast and associated structures. The evidence at hand shows that *Trichogloea* and *Nemalion* are more closely related to each other than to other genera of this family. The criteria in support of the suggested elevation of the subfamily Dermonemeae, which

includes *Dermonema* and *Cumagloia*, to the rank of family are analyzed. It is concluded that there is not at present sufficient ground for separation even as subfamily.

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CYTOGENETICS OF DATURA FASTUOSA L.

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INTRODUCTION[1]

Datura has become classic amongst the cytologists owing to the sustained work carried on for years by Blakeslee and his collaborators. They have been able to isolate nearly 100 distinct and different races of *Datura*, now known as prime types. These races of *Datura* have been shown to have originated principally through segmental interchange. Of 53 prime types, 5 have been found occurring in nature, each differing from the others with respect to particular interchange.

In India, *Datura stramonium* and *Datura arborescens* occur mainly in Sikkim and the surrounding localities. The latter species occurs also in Khasi Hills. Another species, *Datura fastuosa*,[2] closely related to *Datura stramonium*, occurs throughout the plains of India. The two varieties of *Datura fastuosa*, commonly known as the white and black Daturas, are common weeds of waste places throughout India. The two varieties produce forms in nature which differ mainly with respect to leaf, stem, and flower characters. The two strains are distinguished mainly by the presence or absence of anthocyanin pigment in the body of the plant. In the black strain, the stem is black, as are also other parts of the body.

Gates (1942), in his review on the nucleolus and related structures, cited the condition in *D. stramonium* as an exceptional case where, according to Satina, Bergner and Blakeslee (1941), a high number of satellites has been observed. This observation apparently contradicts the theory of numerical correspondence between satellited chromosomes and the number of nucleoli. In the light of the above report, a study of the chromosome-nucleolus relationship in the related species *D. fastuosa* was undertaken.

As has been mentioned already, a large number of forms of this species occur in nature. An examination of these forms is expected to throw some light on the cytological basis of such variation, as will also be evident from the statement made by Dr. W. Burns (1944) in his presidential speech on "Genetics, taxonomy and ecology" which we quote below: "The common

[1] The authors are indebted to Professor R. R. Gates for continued encouragement and for kindly going through the manuscript and to Prof. S. P. Agharkar, head of the department of Botany, for giving facilities to complete this investigation.

Dr. Gates has also made certain minor modifications in the manuscript, and, because of the time which would be lost by transmission to India, has read the proofs.

[2] This is the name commonly used in India for *D. metel*. See Bergner 1943.

weed of waste places, *D. fastuosa*, is found in several forms differing in size and shape of leaf and flower. I should not wonder if a careful study of this species should yield results of a similar nature to those obtained by Blakeslee in his classic work on *D. stramonium* in America."

MATERIAL AND METHODS

The material for the present investigation was obtained from the following: *Datura fastuosa* (black) and *Datura fastuosa*, var. *alba* (white). Morphological variations are met with in both varieties, but in this respect the latter shows a greater degree of variation than the former. The irregularities are mainly manifested in the shape, size, color, and arrangement of the leaves as well as in flower color. The black variety exhibits variation only as regard the different shades of blackness on the vegetative organs and in floral parts. In some, it is present in higher degree, giving the stem a jet black color, while in others the deep purple color is restricted to the nodes. Between the two extremes are intermediate types.

In both the above the fixing of somatic chromosomes in root-tip cells presented considerable difficulties. Root-tips collected from established seedlings did not prove to be fruitful, as they were very slender and as good somatic plates with properly fixed chromosomes could not be obtained from them. Fully imbibed mature seeds were first kept in a cold room having a constant temperature of 74° F; after 2 days, these were placed again in a warm place. This change of temperature was found to give the necessary stimulus for breaking the dormancy, and healthy root-tips were then obtained from the germinating seeds. Several fixatives with various proportions of the ingredients have proved to be unsatisfactory in this case, showing once again why the morphology of the somatic complement could not be made out previously. Blakeslee and his collaborators have obtained good results in fixing the root-tips of *D. stramonium* in a fluid containing 5 parts of 1 % chromic acid and 4 parts of 10 % formalin, but in the present material this fluid has proved to be of little use. Best results were, however, obtained by fixing the root-tips between 12 noon and 2 p.m. in a fluid containing 4 parts of 1 % chromic acid and 7 parts of 10 % formalin. After the usual paraffin embedding of the root-tips, sections were cut 16 μ thick. Premordanting overnight in Lewitsky's fluid (1:1) before crystal violet staining gave best results. For the study of meiotic chromosomes, Flemming's medium fluid gave best results. The paraffin sections of the flower buds were cut 20–22 μ thick and a premordanting overnight in 1% chromic acid became necessary before crystal violet staining. Permanent smear preparations were also made, for which the material was fixed in Belling's modification of Navashin's solution. For the study of nucleoli in the dyad and tetrad stages and in the root-tip cells Feulgen-light-green staining was applied. After a

series of attempts, fairly good results were obtained by first mordanting the preparations overnight in the Belling-Navashin "A" solution before proceeding with the Feulgen-light-green staining. The carbonate mordant (saturated solution of sodium carbonate in 80% alcohol) was used as differentiating solution for the light green stain (see Semmens and Bhaduri 1939, 1941).

The figures were drawn at a table magnification of approximately 3600 times, using a compensating eyepiece No. 18 and a 1.3 apochromatic objective.

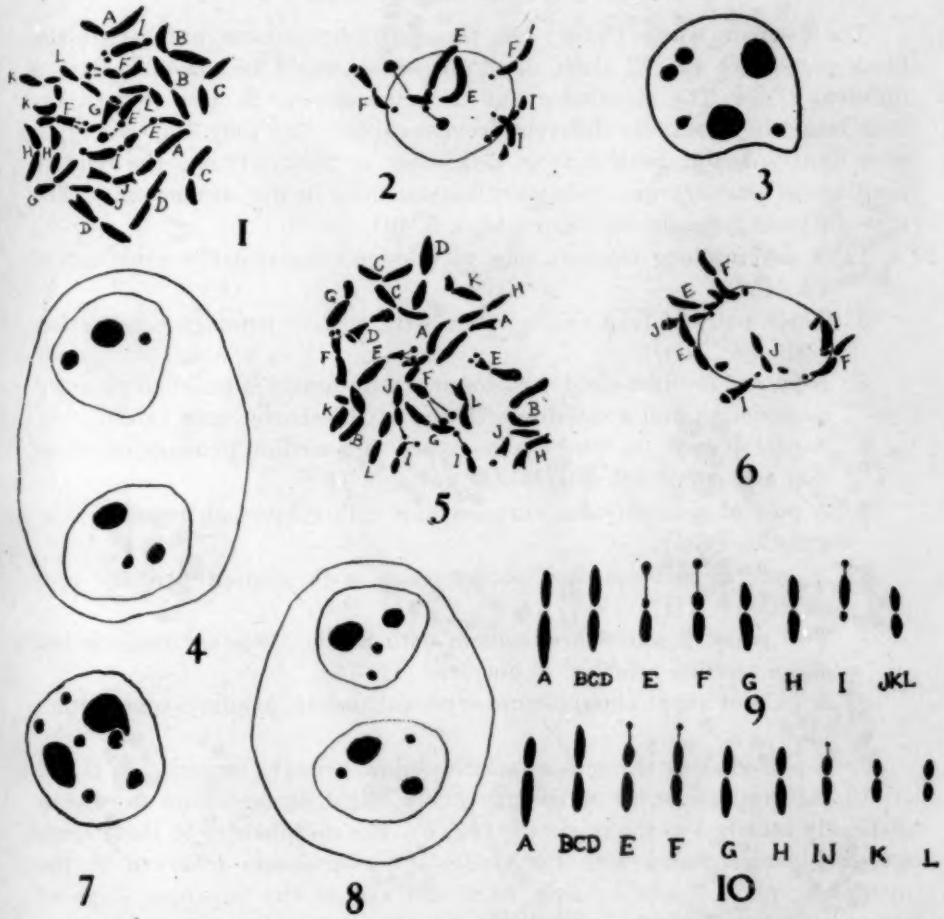
DATURA FASTUOSA (BLACK)

The common black *Datura, D. fastuosa,* has 24 chromosomes in the root-tip cells. The number corresponds with the previous reports made by Belling and Blakeslee (1923), Vilmorin and Simonet (1928), Bhaduri (1938), and Raghavan and Srinivasan (1942). From a critical observation of a large number of well fixed plates, it was found that the chromosomes of this species could be classified into eight different types, clearly distinguishable from each other by their size differences and the relative positions of primary and secondary constrictions. The eight types (figs. 1, 9) are as follows.

1. A pair of long chromosomes with submedian primary constriction (AA).
2. Three pairs of long chromosomes with median constriction (BB, CC, DD).
3. A pair of medium-sized chromosomes with nearly subterminal primary constriction and a big satellite attached at the shorter arm (EE).
4. A pair of medium-sized chromosomes with almost median primary constriction and a satellite attached to one of the arms (FF).
5. A pair of medium-sized chromosomes with submedian primary constriction (GG).
6. A pair of medium-sized chromosomes with median primary constriction (HH).
7. A pair of small chromosomes with subterminal primary constriction and a satellite attached to the longer arm (II).
8. Three pairs of small chromosomes with median primary constriction (JJ, KK, LL).

In the prophase stage of root-tip cells, six chromosomes were seen distinctly attached to the nucleolus (fig. 2). The morphology of these chromosomes corresponded with the nucleolar chromosomes found in the metaphase plate. Six nucleoli were also seen in the telophase stage of root-tip cells, one pair being big, one pair intermediate, and the other pair small in size (fig. 3). Meiosis in the pollen mother-cells was found to be normal. In diplotene and diakinesis, regular chiasma formation and different configurations due to terminalization were evident (fig. 11). The 12 bivalents

seen in metaphase disjoined regularly at anaphase (figs. 12, 13). During the second meiotic division, irregular separation, leading to the formation of 13 and 11 chromosomes at the poles, was observed in a small percentage of mother-cells. Tetrad formation was of the simultaneous type.



FIGS. 1–4. *Datura fastuosa*. FIG. 1. Somatic metaphase plate showing 24 chromosomes including 6 nucleolar ones. FIG. 2. The 6 nucleolar chromosomes attached to the fused nucleolus. FIG. 3. Telophase nucleus showing 3 homomorphic pairs of nucleoli. FIG. 4. Dyad nuclei each containing 3 nucleoli. FIGS. 5–8. *Datura fastuosa* var. *alba*. FIG. 5. Somatic metaphase plate showing 24 chromosomes including 8 nucleolar ones. FIG. 6. The 8 nucleolar chromosomes attached to the fused nucleolus. FIG. 7. Telophase nucleus showing 4 homomorphic pairs of nucleoli. FIG. 8. Dyad nuclei each containing 4 nucleoli. FIGS. 9–10. Idiograms of the two varieties *D. fastuosa* and *D. fastuosa* var. *alba*.

The dyad nuclei often showed the presence of 3 nucleoli in each, one big, one intermediate and the other small in size (fig. 4). It can be stated, there-

fore, that the big, the intermediate, and the small form three homologous pairs. Amongst these the intermediate and the small nucleoli have been seen to remain adpressed on the surface of their respective chromosomes.

DATURA FASTUOSA VAR. ALBA

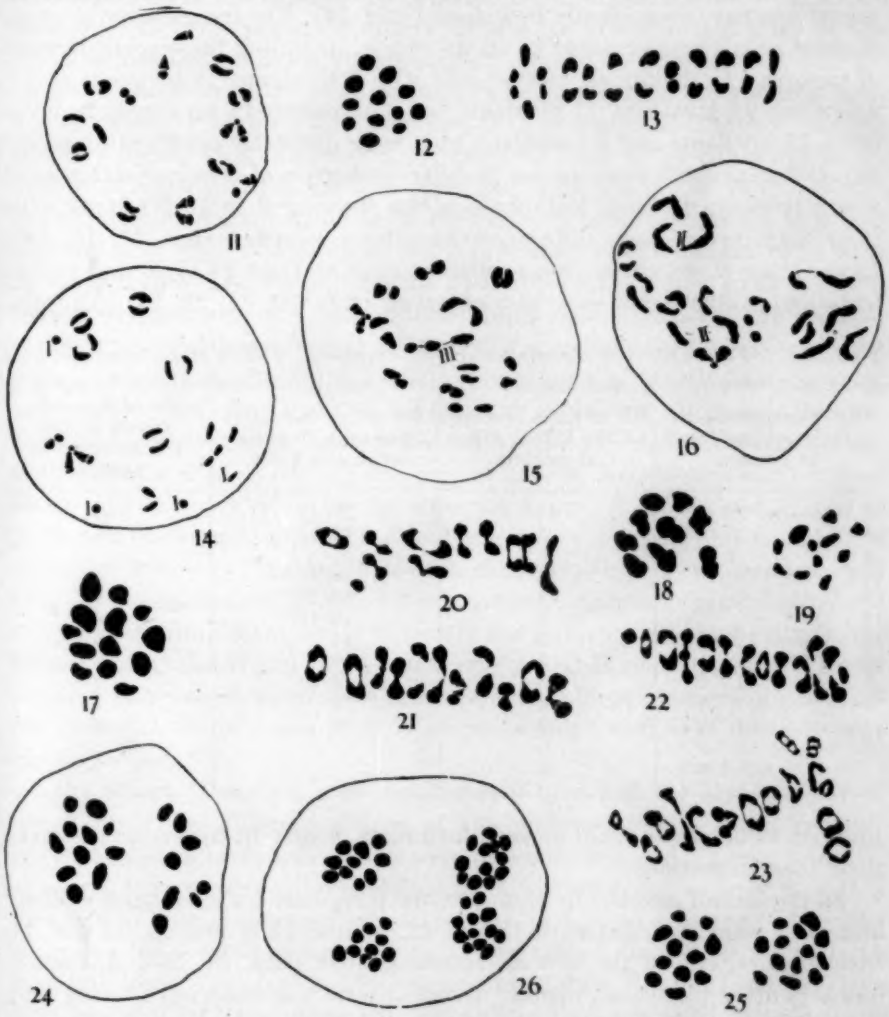
The common white *Datura* has the same chromosome number as the black one. Here the 12 pairs of chromosomes could be classified into 9 different types. The morphology of the chromosomes in this variety has been found to be slightly different from the other. The nine different types were clearly distinguishable from each other by their size and the relative positions of primary and secondary constrictions in the chromosomes. The nine different types are as follows (figs. 5, 10).

1. A pair of long chromosomes with submedian primary constriction (AA).
2. Three pairs of long chromosomes with median primary constriction (BB, CC, DD).
3. A pair of medium-sized chromosomes with nearly submedian primary constriction and a satellite attached at the shorter arm (EE).
4. A pair of medium-sized chromosomes with median primary constriction and a satellite attached at one arm (FF).
5. A pair of medium-sized chromosomes with submedian primary constriction (GG).
6. A pair of medium-sized chromosomes with median primary constriction (HH).
7. Two pairs of short chromosomes with median primary constriction and a satellite attached at one arm (II, JJ).
8. A pair of short chromosomes with submedian primary constriction (KK).
9. A pair of short chromosomes with median primary constriction (LL).

In the prophase stage of root-tip nuclei, eight chromosomes were seen distinctly attached to the nucleolus (fig. 6). The morphology of these chromosomes corresponded with the nucleolar chromosomes observed at the metaphase plate. Eight nucleoli were observed in the telophase stage of somatic nuclei, one pair being big, one pair intermediate, and the other two pairs small in size (fig. 7).

Blakeslee and his co-workers have made a detailed investigation of the meiotic behavior of *D. stramonium*. All the primary trisomics, secondaries, and tertiaries have been found. During the present observation, no prime types or trisomics have been found. Though a large number of plants have been examined, yet all the specimens were found to possess 24 chromosomes in the somatic nuclei. Moreover no ring formation nor the "kite" or "necktie" formations during meiosis, as has been observed by Blakeslee in *D. stramonium* hybrids, were observed.

During meiosis, in the pollen mother cells considerable irregularities of the chromosomes have been observed. Besides the normal occurrence of



FIGS. 11–13. *Datura fastuosa*. 12 regular bivalents during diakinesis, metaphase, and anaphase, respectively. FIGS. 14–16. Diakinetic nuclei showing 10 bivalents + 4 univalents, 11 bivalents + 1 trivalent, and 14 bivalents, respectively. FIGS. 17–19. Metaphase I showing 13, 9, and 11 bivalents, respectively. FIGS. 20–23. Anaphasic separation of 10 bivalents + 1 univalent, 11 bivalents, 10 bivalents + 1 trivalent + 2 univalents, and 14 bivalents, respectively. FIGS. 24, 25. Metaphase II showing irregular distribution of univalents. FIG. 26. Anaphase II showing a distribution of 10 and 10 and 12 and 12 univalents.

twelve bivalents and their normal pairing and segregation, different chromosome numbers ranging from 9 to 14 bivalents were observed. Even in

some of the pollen mother-cells with 24 chromosomes, normal pairing was not observed. So, in diakinesis, ten normally paired bivalents and four univalents have occasionally been found (fig. 14). The irregularity in chromosome number was evident in all the stages, including the second division of meiosis. In diakinesis, mother-cells with 9 bivalents, 10 bivalents and 1 univalent, 13 bivalents, 11 bivalents and 1 trivalent, 14 bivalents, 11 bivalents, 11 bivalents and 1 univalent, etc., were distinctly observed (figs. 15, 16). Different configurations due to different degrees of terminalization were also interesting to note. Metaphase plates showing 9 to 13 bivalents with their characteristic size differences have been recorded (figs. 17, 18, 19). In anaphase stage, chromosome numbers ranging from 19 to 26 and univalents and multivalents were also observed (figs. 20, 21, 22, 23). The dis-

TABLE 1. *Different types of segregation during anaphase I & II in D. fastuosa var. alba*

Diff. chromosome No. & their pairing found in 1st. div.	No. of times observed in 100 P.M.C. taken at random	No. of univalents at one or both poles during 2nd. div.	No. of times observed in 100 P.M.C. taken at random
12 bivalents	43	12–12	50
13 "	8	10–11	13
9 "	3		
11 "	14	8–11	3
10 "	6	12–11	4
14 "	3	10–10	3
10 bivalents + 1 univ.	3	11	15
11 " + 1 "	3	10	5
9 " + 1 "	5	8	2
12 " + 1 "	4	13	4
10 " + 4 "	5	14	1
10 " + 2 " and 1 triv.	3		

junction of bivalents could be examined more clearly in the smear preparations than in sections.

In the second meiotic division also, the irregularities were quite evident and cases were recorded with 13 and 12, 10 and 11, 8 and 11, 12 and 11 chromosomes, etc. in the two metaphase groups (figs. 24, 25). Abnormal numbers after the second meiotic divisions were also observed passing into the tetrad stage (fig. 26). In the present investigation an attempt has been made from a large number of observations to find out the average frequency of the occurrence of different chromosome numbers in the P.M.C.'s resulting from such irregularities. The observations are presented in table 1. From this table it will be evident that nearly 50 per cent of the P.M.C.'s bear irregular numbers.

It is interesting to note that all the irregular types could be seen sometimes within the same anther lobe.

It may be concluded from the above observations that some change in the premeiotic divisions must be responsible for the production of P.M.C.'s with abnormal chromosome numbers. It is quite probable that some of the chromosomes fail to divide at this stage. This will lead to increase and decrease of chromosome numbers at the two poles and thus mother-cells with higher or lower chromosome numbers than the normal haploid number will be formed. If it is assumed, for instance, that of 24 chromosomes 21 undergo normal division and the remaining 3 do not divide fully, i.e., the two chromatids remain attached by their centromere, move to one pole and separate later, then this will lead to the formation of one daughter-cell with 21 chromosomes and the other with 27 chromosomes. Such irregular premeiotic division will, as a rule, produce duplication of some of the chromosomes in some mother-cells and deficiency of some other chromosomes in the same number of mother-cells. Thus the occurrence of multivalents in some and univalents in other P.M.C.'s can easily be explained. Further, if the nondividing chromosomes be equally shared by the two daughter-cells, abnormalities in the P.M.C. bearing the normal $2n = 24$ chromosome number may result. For example, if of 24 chromosomes 4, viz., A, B, C, and D, fail to divide during anaphase, and if A and B move to one pole and C and D to the other, then one of the daughter-cells, though having 24 chromosomes, will have the duplicated sets of A and B chromosomes and the other daughter-cell will have the duplicated set of C and D chromosomes. Consequently in the former case C and D and in the latter case A and B respectively will be deficient. Furthermore, occurrence of univalents in large numbers in a nucleus also indicates the presence of extra chromosomes which have failed to pair during meiosis.

The tetrad formation is of simultaneous type and the arrangement of tetrad nuclei in different planes of the mother-cell was quite evident. Irregularities in tetrad formation were also observed and "linear tetrads" of spores have occasionally been found. It was observed in some P.M.C.'s that late in the season variations in temperature were accompanied by the occurrence of several, as many as eight, free nuclei, indicating the extreme susceptibility of the plant to temperature fluctuation. Similar instances have been reported in *Hyacinthus* (de Mol 1923), *Triticum* hybrids (Kihara & Lilienfeld 1936), *Kniphofia* (Moffett 1932), *Tradescantia*, and *Gaura* species (Bhaduri 1941, 1942a).

The dyad nuclei show 4 nucleoli in each nucleus, one being big, one intermediate, and the other two small and similar in size (fig. 8). The two small nucleoli were seen closely appressed against the respective nucleolar chromosomes. It can be stated therefore that the big, the intermediate, and the two small nucleoli form 4 homologous pairs.

Pollen grains are of the same type as found in the black variety, but in

this variety pollen grains with different sizes and shapes (small, medium, and large), showing also partial abortion, were not infrequently met with. The percentage of abortive grains (15 to 18) was not so high as the irregularities of chromosomes in P.M.C. (as many as 50 per cent of the mother-cells) during meiosis would suggest (cf. table 1). Presence of pollen grains of varying sizes can be explained on the basis of chromosome deficiencies and balance (Darlington 1929, Bhaduri 1942b). Attempts were made to find out the time of division of the pollen grains but satisfactory results have not yet been obtained.

DISCUSSION

Although extensive work has been done in *D. stramonium* by Blakeslee and his co-workers, very little is known about the allied species *D. fastuosa*, the common weed of waste places in India. As has been pointed out in the introduction to this paper, Gates (1942) in his review has mentioned that in *D. stramonium* a high number of satellited chromosomes, as many as seven in the haploid plant, have been found by Satina, Bergner and Blakeslee (1941). The latter authors have, however, not examined or counted the number of nucleoli in this species. In *D. fastuosa* it will be found from the present investigation that an exact correlation exists between the maximum number of nucleoli and the number of satellited chromosomes. The two varieties of *D. fastuosa* differ, however, from each other in the number of nucleoli and the number of satellited chromosomes present, the maximum number of nucleoli and nucleolar constrictions found in the somatic complement in the two varieties being 6 and 8 respectively. A corresponding number of nucleoli in the telophase stage of root-tip cells, in dyad and tetrad nuclei has also been found. This observation is also confirmed from the number of attachments of chromosomes to the fused nucleolus of the somatic prophase stage. It is interesting to note that the morphology of the somatic chromosomes, excepting the satellited ones, is very similar in the two species *D. stramonium* and *D. fastuosa*.

In view of the above observations, it may be suggested that a critical re-examination of *D. stramonium* with respect to the maximum number of nucleoli and satellited chromosomes is desirable.

It has been generally assumed that a true diploid species should have a pair of homomorphic nucleoli in the somatic nuclei (Bhaduri 1943). Increase in the number of nucleoli or the presence of size differences between the nucleoli in a species has been interpreted to be due to numerical and secondary polyploidy, segmental interchange between nucleolar and non-nucleolar chromosomes (Bhaduri 1943), and fragmentation of satellited chromosomes (Bhaduri & Bose 1946). Both *D. stramonium* and *D. fastuosa* are generally taken to be true diploid species. Previous observation in *D.*

stramonium and the present work on *D. fastuosa* do not give evidence as to the secondary polyploid nature of the two species. No secondary association between bivalents, a characteristic feature of secondary polyploid plants, has been observed in these two species. There is evidence, however, to show that considerable structural changes of chromosomes have taken place in *D. stramonium*. According to Bergner, Satina and Blakeslee (1933) and later work (Blakeslee et al. 1940), there are 96 prime types of *D. stramonium*, each differing from the parent standard type with respect to one or more interchanges. If such interchanges are involved between nucleolar and non-nucleolar chromosomes, then the origin of a high number of secondary constrictions and nucleoli is expected (McClintock 1934, Bhaduri 1942a).

It will be interesting to know how the prime types differ from the standard *D. stramonium* with respect to the maximum number of nucleoli and satellited chromosomes. Unlike *D. stramonium*, in both the varieties of *D. fastuosa* no evidence of translocation has been obtained from the behavior of meiotic chromosomes in the P.M.C., since no ring of four or "kite" or "necktie" type of formation during meiosis has been observed. In the white strain, as described in the text, a very remarkable irregularity in as many as 50 per cent of the P.M.C.'s during meiosis has been recorded. It has been pointed out, that in the premeiotic mitosis, failure in the division of one or more chromosomes and their subsequent inclusion in the P.M.C. nucleus often leads to the production of spores having either higher or lower numbers of chromosomes than the usual gametic number. Random union of such gametes can not only produce plants having higher or lower chromosome numbers than the normal diploid number ($2n = 24$), but otherwise than in *D. stramonium* we find here a possibility of having species with the normal diploid chromosome number $2n = 24$, at the same time having one or more chromosomes duplicated. If nucleolar chromosomes are involved in such abnormalities then an increase or decrease in the number of nucleoli and satellited chromosomes may easily result. On the basis of this hypothesis, the formation of a ring of four and multivalent formation in *Datura* can also be explained as due to duplication of chromosomes by irregular premeiotic mitosis without assuming previous translocations, as found in many prime types of *D. stramonium*.

It is remarkable that although spores with such irregular numbers have been found in the white strain, *D. fastuosa* var. *alba*, trisomics or plants with higher or lower number of chromosomes appear to be rare. Further, when we assume duplication of one or more chromosomes in the white strain we should also expect multivalent formations during meiosis. During the present observation, however, although a good number of plants have been

examined, the percentage of multivalent formation during meiosis has always been found to be very small.

Some explanation of the physical basis of the origin of different forms of *D. fastuosa* can be obtained in the light of the observations made regarding the production of spores having different chromosome numbers and particularly of the origin of spores having the same chromosome number but of different genotypes. As is evident from table 1, about 50 per cent of the P.M.C.'s bear an irregular number of chromosomes. Furthermore, the percentage of abortive grains has been found to be only 20 per cent approximately. Therefore it is quite evident, that about 30 per cent of these spores remain viable with irregular numbers of chromosomes. Whether these different forms of *D. fastuosa* occurring in nature are distinct phenotypes, breeding true to character, or different ecological strains, remains to be proved by breeding experiments. Preliminary experiments in this direction have already been started. In the black strain, where fairly regular behavior of chromosomes has been observed during meiosis, we find also variation, though on a comparatively smaller scale.

SUMMARY

The two varieties of *D. fastuosa*, the black and the white, a common weed of India, differ from each other with respect to the presence or absence of anthocyanin pigment in the body of the plant. The white variety shows a wider range of variation as compared to the black one. A comparative idiogram study of the two varieties has revealed a close similarity between the two, except that in the black variety there are three and in the white there are four pairs of chromosomes with nucleolar constrictions. Corresponding to this, there are three and four homologous and homomorphic pairs of nucleoli present in the two varieties respectively. The present observation is in conformity with the theory of numerical correspondence between the maximum number of nucleoli present in a species, and the total number of secondary constrictions present in the chromosome complement of that species. A re-investigation of *D. stramonium* in the light of the present investigation has been suggested.

In both the varieties normal pairing of twelve pairs of chromosomes has been observed. No evidence of secondary association between bivalents could be obtained. Excepting occasional trivalents, ring formation or other multivalent formations have not been found. While a fairly normal meiotic behavior is present in the black variety, in the white variety a very interesting condition has been observed. Some chromosomes fail to separate in the premeiotic mitosis with the result that mother-cells showing higher or lower chromosome numbers ($n = 9, 10, 11, 12, 13, 14$) than the haploid number are formed. This irregularity leads to duplication or deficiency of some of

the chromosomes and consequent univalent and occasional multivalent formations. It has been shown how this type of irregularity may not only lead to the formation of gametes having unequal chromosome numbers but also to gametes having the normal haploid number but of different genotypic constitution. Random and successful union between such gametes provides an explanation of the cause of origin of trisomics, tetrasomics, $2n-1$, and other types, as well as of plants showing the normal diploid number but of different genotypes. These may also differ phenotypically. The wide range of variation found in this variety should be analyzed by breeding experiments in the light of the present observations.

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NOTES ON THE VEGETATION OF SIERRA SUROTATO IN NORTHERN SINALOA

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Sierra Surotato rises in great form up from the coastal plain in northern Sinaloa. Those who ride the rails may look out northeastward in the latitude of Guamuchil (25° 30″ N) and see its blue-distant hulk, often crowned with clouds, and, if the atmosphere is clear, see also the rugged broken rims of rocks and numerous penetrating canyons. I beheld it so in the spring of 1940 and thereafter that distant beauty and the lure of unknown and unsought plants secreted in the mountain fastnesses led me back to visit it in 1941 and again in 1945.

The top elevations of Sierra Surotato range from 6500 to 7500 feet above sea level. It is a coastal outpost of the Sierra Madre Occidental, the main axis of which is carried through Sierra Mohinora in Chihuahua about 40 airline miles eastward. Surotato's position is comparable to that of Sierra de Alamos, classic plant locality in southern Sonora, but its bulk is much greater. Generally in Mexico the inhabitants have local names for mountains and neglect general or inclusive appellations for mountain systems and this is true of the Surotato complex. Sierra Surotato is composed, from north to southeastward, of Sierra Ocurahui, Sierra Surotato, and Sierra Monterrey. The situation is shown on the accompanying map (fig. 1), which should not be assumed as accurate in detail. Since this sierra is not named on any maps I have reviewed, it can serve until measured detail is supplied. Its position is well indicated on the one-to-a-million Map of the Americas, Culiacan quadrangle, published by the American Geographic Society in 1935. My aneroid readings showed the highest peak, Surotato, to be 500 to 600 feet higher than the 2192 m. given on that map. The system is 40 to 50 miles long NE to SE and variously dissected by deep precipitous canyons and intricately ramified by radiating ridges.

The core of the mountain is granitic. It was observed as the common rock in the Tamiapa foothills on the southeast and again through the foothills on the west as far out as San Joaquin. Laid over the granite through high and low elevations are various lavas of various ages and extent. In the higher elevations are both massive and laminated sedimentaries, highly deformed and often metamorphosed. These are strikingly exposed atop Sierra Monterrey (fig. 2). Mainly, however, it is the volcanics that form the rugged cliffs and peaks of the higher elevations, some strongly calcareous as the Peñascos de Pucheros.

Sierra Surotato is relatively wet as attested by the rather numerous epiphytic bromeliads, orchids, lichens, mosses, and the preponderance of mesophytic and tropical species. The rainfall station nearest to Surotato is in Badiraguato, lying in a foothill valley 30 to 40 miles southeast. Its annual average is around 30 inches. That of Sierra de Alamos, about 100 miles north of Surotato, in Sonora is between 20 and 25 inches annual average. With

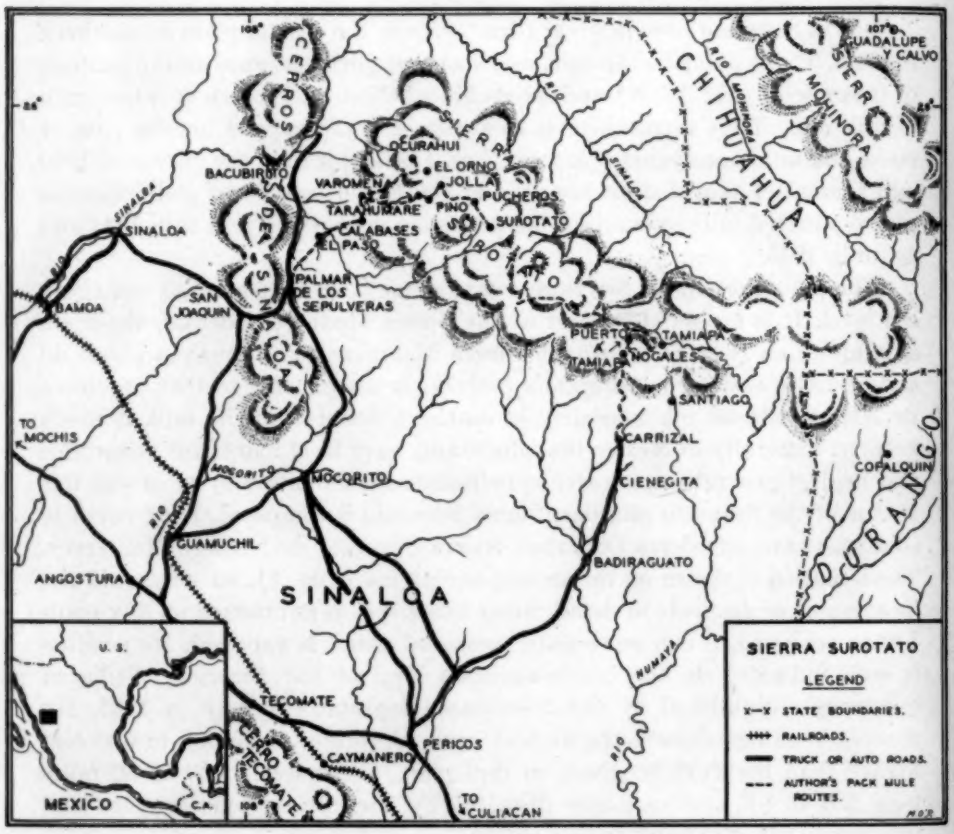

FIG. 1. The Sierra Suratoto region. Scale 1: 225,000.

these records (1) and the indications from vegetation we can estimate the higher wetter elevations of Sierra Surotato to exceed 40 inches annually. The season is binary; rains from May to February with a mild drought period in the fall, and long drought through the spring. Occasionally spring rains occur, however, as in March 1940 when it rained heartily for a week, much to the surprise and consternation of the collecting botanists. The copious summer rains are convectional in type, of tropic origin; the more

violent storms are known as "chubascos." The "equipatas" (< L.—even feet) are the light even rains of winter.

White clouds that the lowlanders see investing the heights of the mountain are only fog to the mountaineers. This reduces the incidence of sunlight considerably over that due to rain clouds alone, since it may appear both preceding and following diurnal rain hours, and may also occur as ground fog lying in the highland valleys in the early morning. The foggy forest is gloomy and dripping, festooned with hanging mosses, cool, and inhabited

FIG. 2. Sierra Monterrey forested with pine and oak; looking north, March 1940. The anticlinal massive sedimentaries dip westward.

only by shade-tolerant species on the northern slopes. Fog is undoubtedly a chief factor in supporting the rich communities of epiphytes and the varied forest of tropical nature.

Snowfalls are said to be of almost yearly occurrence at Ocurahui and throughout the higher elevations. As reported by the natives, they are light, lie but a little while, and fall in January and February. Yet orchids and *Tillandsia* grow in these elevations of snowfall (fig. 3), suggesting an unexpected tolerance to cold. However, plants were observed in rather spotted occurrence, indicating that they are restricted in these higher elevations to warmer spots conditioned by local features of terrain. The area of snowfall is within the pine zone.

The vegetation of Sierra Surotato has been markedly disturbed by man in a small percentage of the area. Regular planting and cattle are limited to the areas of less rugged terrain. Milpas of beans, squash, potatoes, wheat, and maize, and small orchards of peaches, quinces, apples, and oranges are scattered through the mountain adjacent to the small settlements. There is a small planting or two of coffee in Canyon Tarahumare, the most northerly occurrence of this crop in North America. The opium poppy, *Papaver somniferum*, is scattered in small plots, spring-grown, often following wheat. Though the poppy is dry-farmed, it is preferably planted where it can be irrigated by diverting the small mountain rills. The uncertain spring rains may or may not be sufficient for a good yield of gum. It is a contraband product providing the mountaineers with a ready cash crop. Because of this they are suspicious of strangers and I was once threatened with indefinite detention.

Transient milpas through the past two decades have made serious mutilations of the mountain cover. This is conspicuous through the Canyon de Tarahumare, where even precipitous rocky slopes of 40° to 60° inclination are cut over for only the one or two crops of maize they will produce. Increase in such cuttings is correlated with increase in local population. These natives refuse offers of more arable land in the coastal plain because they prefer to live in their mountain environment, believing that they would suffer and fall ill in the hot lowlands. They are poor and subsist upon an incipient agriculture which would be inadequate for any modern society. The native trees are also felled for local fuel and lumber.

The rancho, Los Pucheros, is remarkable for the degree of comfort and orderliness with which it has been maintained. This is entirely due to an elderly bachelor, now crippled, native of the mountain, who admires and can name every state in our union, although he has spent his whole life upon Surotato. In his pine log buildings with shake roofs he quarters summer vacationists from the hot lowlands. Mostly they make the laborious trip by mule, but airplanes have been landed at the neighboring rancho of El Horno several miles away. The landing strip is a mountain meadow atop the sierra. Landings, however, should not be attempted without first ascertaining the condition of the field.

The natural vegetation of Sierra Surotato is thriving, unusually complex, and covered by slope forests of highly mixed dominants, largely deciduous in the lower elevations, pine-dominated in the upper. The more arid element is found in the Short-tree Forest formation (2) of the lower slopes. Upward in the wetter elevations, 2500–4500 feet, are many intruding tropical species. Here the formation is more nearly a tropical deciduous rainforest with an increase in the density of shrub species over that found in typical Short-tree Forest. Tropical mesophytes are best represented in the

FIG. 3 Stand of young oaks at about 6000 feet elevation near Ocurahui, showing epiphytes of *Tillandsia inflata* and moss. FIG. 4. The valley of Ocurahui in September 1941. Elevation about 6000 feet. *Cornus excelsa* lines the cut-in creek meandering through the meadow. Scattered pines mix with several hardwoods.

deep canyons, following upwards high into the oak zone. The nearly pure oak forest grades into Pine-oak Forest between 4000 and 5000 feet. Madroño, *Arbutus*, is common at 6000 feet and above. The numerous communities and the heterogeneous associations are indicated in the following annotated list of collecting localities.

Varomena, elev. 1000 feet. This village of a few hundred people lies in the foothills at the northwest end of Sierra Surotato and perhaps 70 miles northward from Guamuchil. It is the end of the crude truck road. It is rather typical of the foothill valley country thereabouts. Much of the virgin cover has been cut over and used for milpas or over-grazed. Some of the hill slopes contain small or large areas of but little disturbed woodland of Short-tree Forest composition (2). A messy, aggressive, scrubby cover is commonly adventive upon fallowing milpas and might well bear the name of subtropical scrub of writers, who apparently have not always recognized its real transitory position in the development of the vegetation. An old man in Varomena told me that formerly "encinos" or oaks grew upon the hills above Varomena and that the original "monte" had been cut away. However, some relics of the virgin forest cover remain and one which was visited lay about two miles northeast of the village on a northern slope. It showed a good forest growth with a general stature of mature trees about 30 to 40 feet, dominated by a mixture of drought-deciduous species. The relatively large mesic leaf (average area perhaps 75 cm.^{2}) was more common than the smaller pinnatifid type as represented by the *Mimosaceae* and *Burseraceae*. The former type was generally palmately divided as in some of the common dominants such as *Ceiba acuminata*, *Cochlospermum vitifolium*, and *Tabebuia palmeri*. *Ipomoea arborescens* var. *pachylutea* was one of the large-leaved dominants and formed large trees about 40 feet in height with a trunk diameter of two feet or over. It is one of the common tree species through the lower elevations of Sierra Surotato, occurring both in virgin cover and on cut-over areas. Four different species of *Bursera* were found in this segment of forest, some with finely cut leaflets. A few vines were climbing trees and *Cissus rhombifolia* was collected. Secondary shrubbery was only thinly present and little difficulty was found in getting about through the trees. On the whole its composition is typically Short-tree Forest except for the light infusion of oaks, which may be explained by an original adjacent formation of Savanilla occurring transitorily through the region and previously reported near Sierra Tacuichamona (3).

Savanilla is a minor member of the foothill vegetation. Arborescent species from Thorn Forest, Short-tree Forest, and Oak Forest occupy the rocky outercroppings, where rapid water carriage of weathering products does not allow appreciable soil formation, while turf grasses cover the intervening evener slopes whose soil is a coarse mixture of gravels and clay. The basic

rock extrusions are commonly the heights of volcanic pediment which underlies the shallow surface soils. The topography of such terrain is much less precipitous than the Barranca Region proper and the best development of Savanilla is in rolling or slightly broken topography. Those who regard grassland as a late and more highly evolved feature in the development of organic covering are here happily sustained in this striking illustration. The arborescent species are limited to the physiographically younger part of the terrain, the degrading rocks, while grasses monopolize the aggrading miniature plains and gentle slopes. The arborescent mixtures often run strongly to the Cuajiotal type, but not to the degree described in southern Mexico (4), and as observed elsewhere in this formation along the west coast. The trees are preponderantly drought-deciduous and short-statured.

Las Mesas, elev. 3000 feet. Las Mesas, a half day's pack trip southeastward from Varomena, is a rancho with two or three houses in what may be called bench land about half way up Sierra Surotota. Small mesas support a grass cover with oaks and Short-tree Forest species upon adjacent slopes and canyon origins. The mesas themselves are trifling but interesting instances of atypical savanna. Local physiographic features and climate have united to effect unusual mixing of dominant species and with this there is an inherent similarity to the lower lying Savanilla.

Striking associations are the rocky glades and ridges of *Ipomoea–Bursera* stands, the littered rocky slopes of pure oaks on northern slopes, and a highly mixed deciduous forest on southern exposures. The tall slender *Bursera arborea* is found in canyon origins. Herbs and shrubs are plentiful and highly mixed in moist situations with highland and lowland species overlapping.

The soil is shallow, volcanically derived, and the run-off rapid. Pines are lacking. The climate is most equable. The rocky terrain and shallow soils appear to be the chief factors in limiting the vegetational configuration, resulting generally in bringing together a number of plants that can gain foothold within their respective limits in amounts of soil needed. Dwarf thick-stemmed Burseras only 4–6 feet high were found growing out of the crevices of bare rocks, while their normal-statured brothers stood by, 15–20 feet high, in shallow pockets of soil.

Fog is not prevalent at this elevation and is correlated with a relative lack in the epiphytic population. The pendulous lichen is lacking altogether, orchids and *Tillandsia* infrequent.

Varied grass stands occupy most level spaces and gentle gradients. Both turf and bunch grasses are found on open slopes, while *Paspalum plicatum* was found thriving in moist depressions together with *Cyperus* and *Cuphea procumbens*. The natives consider this grass good forage. Along the trail up to Las Mesas beautiful specimens of *Lemaireocereus montanus* were observed.

Ocurahui, elev. 6000 feet. Ocurahui is a scattered settlement of four or five houses about a day and a half by mule eastward from Varomena. It is a delightful, park-like valley cradled in the pine lands on the northern end of Sierra Surotato (fig. 4). A shake-roof storehouse was hospitably donated by Don José Rochas, the loquacious, intelligent, polygamous, and most influential man of that part of the mountain. The storehouse provided the mosos and me adequate shelter from daily summer storms, facilities for drying presses over a smoking fire, and except for the piggy odor emanating from a pen at one end was entirely pleasant. Edible food, however, was scarce and I knew hunger long before my stay was over. The rolling valley was rich in meadow and slope grassland, orchards harbored native herbs, and a dashing creek came out of the surrounding forest, bubbled through the celestial valley, and went out into the woods again, falling ever into the deep canyons below. Gentle valley slopes are exceptional in the mountain. It is mostly quite broken and rugged; stout legs and good lungs are needed for getting about.

There are very few pure stands of pine about Ocurahui. The dominant forest type is a mixture of pine and oak, as outlined in the following list, arranged approximately in their order of dominance.

Pinus macrophylla Engel.
Pinus ayacahuite K. Ehrenb.
Pinus oocarpa Schiede.
Pinus lumholtzii Rob. & Fern.
Pinus pseudostrobus Lindl.?
Quercus epileuca Trel.
Quercus pallescens Trel.
Quercus durifolia Trel.
Quercus gentryi Mull.
Quercus candicans Nee.
Quercus endlichiana Trel.
Quercus pennivenia Trel.

Pinus macrocarpa and *P. oocarpa* appear to have the greatest altitudinal ranges. *P. lumholtzii* is common, while *P. pseudostrobus* (?) is scarce, scattered, and not found at lower elevations. On the whole the pine growth is small and only small timber was observed. It is employed for local building, for torches, and for kindling by the natives. Oak is preferred as firewood. *Quercus epileuca* is most abundant upon the moist shady slopes, growing with long straight boles as high as the smaller pines, 15–20 meters. *Q. pallescens* is more common over all types of exposures. *Q. gentryi* is not uncommon and forms rather densely leafy crowns with its narrow leaves. *Q. pennivenia* is restricted to sparse stands on arid slopes with the old trees low and rounding, 6–8 m. high, the branches low and the whole forming a wide symmetrical big-leaved tree. *Arbutus xalapensis* was collected on Sierra Monterrey and often takes its place with the pines and oaks.

In the high moist cool canyons the pines and oaks are often replaced by other more mesophytic trees, nearly all of which are shade-tolerant. Among them are *Styrax argenteus, Ostrya virginiana, Cornus disciflora,* and along the streams *Cornus excelsa, Garrya laurifolia,* and *Magnolia schiedeana.*

The shrub population is rich in species, *Rhus allophylloides* and *Rhamnus* sp. are both shade-tolerant deep-canyon dwellers, the latter forming riparian colonies. *Crotalaria mollicula* is an herbaceous subshrub and it forms extensive colonies upon high moist shade slopes with the low herbaceous *Erythrina montana* infrequently infiltrating. *Triumfetta chihuahuensis* is another canyon shrub, while other members of this tiliaceous genus are common on the more open slopes mostly at lower elevations. Herbs are present in numerous species, occurring only very thinly under pine stands, but occasionally form-

FIG. 5. Slope forest of oak in the Quebrado de Mansana, September 1941. View overlooks tops of *Platanus* trees growing along stream in canyon bottom. *Lysiloma watsoni* in right foreground and a few scattered along gullies with the oaks.

ing a floor covering under mixed forests with such species as *Ipomoea decasperma*. The grass cover is generally quite sparse or lacking under the forest, but thin stands of *Aristida schiedeana* and *Muhlenbergia rigida* occur under the trees.

Quebrado de Mansana, elev. 3000–5000 feet. This is a deep canyon between Ocurahui and Las Mesas with an abandoned mine and a few ranchos scattered along the bottom. The north exposures in the upper extents are covered with pines that grade downward into oaks. The southern exposures

are dominantly oaks; *Quercus chihuahuensis, Q. tuberculata, Q. albocincta.* The main canyon is lined with tall aliso trees, *Platanus racemosa* var. and with them in the tributary canyons is a strong infusion of barranca and lowland trees; *Ceiba acuminata, Ipomoea arborescens pachylutea, Oreopanax peltatum, Inga eriocarpa,* intermingled with highland species. Shrubbery is rampant under the slope forests and mixed with numerous species; bush-like composite types, wand-like shrubs as *Mimosa* spp. and *Perymenium stenophyllum,* and the small graceful *Triumfetta goldmannii,* and the larger tree-like forms as *Eysenhardtia reticulata, Calliandra gentryi,* and *Heliocarpus* sp. with broad leaves. Altogether they form a closed cover rather difficult to negotiate on the precipitous slopes. On the moist slopes and bottoms the herbs form a thick carpet and are prevailingly shade-tolerant, broad-leaved, turgescent types. Grass species are only scattered here and there in these wooded moist slopes and vary greatly in habit.

The south exposures are essentially more arid and less shrubby. *Eriosema* and coarse grasses are typical of the low well-spaced cover on the more rocky areas, with grasses becoming dense on moist slopes with more soil. The prevailing type of leaf is stiff and harsh in both monocots and dicots, as pointed out as characteristic for the Oak Forest belt of northwestern Mexico (3). Shrub thickets are generally lacking though individual shrubs occur. The herbaceous flora is rich in species, variously adapted to the small moist gulleys or the open arid slopes.

Quebrado de Tarahumare, elev. 2000–5000 feet. The canyon de Tarahumare is the greatest western dissection of Sierra Surotato and contains the most settlements. Its vegetation, therefore, has been more disturbed than that of the Quebrado de Mansana, but in its several precipitous tributaries is still found a great deal of virgin forest. The make-up of the vegetation is essentially similar to that noted for Mansana, but it does contain species rare or lacking in Mansana. It is generally true of Sierra Surotato that every major canyon has its own floristic make-up. As one passes from one canyon to another new plants meet the eye. This is a characteristic of tropical floras, which in North America reach their climax in the exceedingly rich flora of Costa Rica, where within an area of 18,400 sq. miles over 6000 species of plants have been catalogued (5).

The canyons of Sierra Surotato are the meeting places of temperate and tropical genera. The following list shows several plants not previously known as associates or as having over-lapping ranges.

Temperate	Tropical
Ostrya virginiana (Mill.) Koch.	*Distylium* sp.
Platanus racemosa Nutt. var.	*Magnolia schiedeana* Schlecht.
Juglans major (Torr.) Heller	*Brosimum alicastrum* Swartz.
Prunella vulgaris L.	*Garrya laurifolia* Hartw.
Aquilegia aff. *skinneri* Hook.	*Picramnia antidesma* Swartz.?

Agrimonia striata Michx.
Corydalis montana Engelm.
Lathyrus leucanthus Rybd.
Viola neomexicana Greene.
Sisyrinchium arizonicum Rothr.
Cnidosculus angustidens Torr.
Mimulus verbenaceus
Psittacanthus macranthmus Eichl.
Styrax argenteus Presl.
Bocconea arborea Wats.
Chamaedorea sp.
Calathea aff. *cyclophora* Baker.
Maranta arundinacea L.
Klaprothea mentzeloides H.B.K.
Zeugitis latifolia Hemsl.
Cupania glabra Sw.

The records of plants collected on Sierra Surotato not only contribute to our knowledge of the distribution of northwest Mexican plants, but are significant in interpreting the evolution of the floras of southwestern North America. In this mountain is a varied number of plants whose observed or recorded distributions are discontinuous in the latitudes of the desert-tropical transition. A few examples are: *Ostrya virginiana, Agrimonia striata, Lathyrus leucantha, Corydalis montana,* and the disjunct *Platanus racemosa,* all of northern origin. Most of such distributions appear to be relics representing Pleistocene adventives established during glacial epochs, when the cold front was farther south. Examples of plants whose distributions appear to be, or are established as, discontinuous area: *Brosimum alicastrum, Distylium* sp., *Chamaedorea* sp., *Calathea* aff. *cyclophora, Klaprothia mentzeloides, Athyrocarpus leiocarpus, Bocconea arborea, Zeugites latifolia,* and *Cupania glabra.* Such distributions may also be explained on the basis of climatic succession during Pleistocene periods, as relics in the more sheltered canyons left from populations retreating before the south moving cold fronts.

The endemics of the desert-tropical transition, which are many, together with other species of broad tolerances form the great bulk of the flora. They are the great, rugged, plastic element that moved down from the Southwest into Mexico when the arid and the arctic dominated the north temperate (6, 7). Most of the component species, either quickly or slowly, are persistently aggressive in occupying new coastal lands (e.g. the rising coast) and abandoned clearings (2).

The theory of plant migrations under the duress of migrating climatic zones, due to geomorphism and varying sunlight during geologic ages, may be of great value in explaining the development of plant distributions. Certainly no field could be more fruitful in source material than the long desert-tropical transition flanked on the west by the great sea and on the east by the Sierra Madre Cordillera. Here variable temperature operated with altitudinal zonation to give latitudinal movement as well as the more universal longitudinal migration to the mobile biota. Before historical interpretations of distribution can have accuracy, however, an intimate knowledge of plant placements is necessary. On the whole the vegetation of Sierra Surotato and environs is of desert-tropical transition, with a tropical element more preponderant than a temperate one. This is just below 26° north latitude, some 150 miles above the Tropic of Cancer.

In conclusion the following outline approximates the actual zonation of the major forms of vegetation on Sierra Surotato and adjacent coast.

Pine Oak Forest	4500–7000	feet elev.
Oak Forest—Grassland	2000–4500	" "
Tropical Montane Forest	3000–4500	" "
Short-tree Forest	500–3500	" "
Thorn Forest	0–1000	" "

These major zones of vegetation are not sharply divided, because of the interpolated and intergrading tropical element. It is represented by both the Short-tree Forest and the Tropical Montane Forest, the latter stratigraphically equivalent to the oak belt, the presence of one or the other conditioned by exposure, soil, and local climate as affected by terrain. The Tropical Montane Forest occupies the equable central elevations where the higher precipitation is retained by good soil and evaporation relatively reduced by the cloud layer. It is limited to the canyons, while the dispersed oak formation is general over the slopes. On the whole this zonation is remarkable and will be analyzed in an early paper.

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GLYCERIA PALLIDA AND G. FERNALDII

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Glyceria Fernaldii (Hitchc.) St. John, based on *G. pallida* var. *Fernaldii* Hitchc., was first proposed as a species by St. John (Rhodora **19**: 75. 1917), and was taken up as a species in the *Manual of Grasses of the United States,* page 94, under the name *G. neogaea* Steud. Mrs. Chase subsequently showed that the type of *G. neogaea* was *G. stricta* (Am. Jour. Bot. **24**: 33–34. 1937).

The characters pointed out by St. John were to a large degree concerned with size of parts; *G. Fernaldii* was described as having a smaller grain then *G. pallida,* shorter anthers, smaller spikelets with fewer florets, more divergent or reflexed branches of the panicle, smaller leaves and lower culms. Table 1 shows how four of these characters appear on 19 sheets in the Herbarium of the University of Wisconsin and the Milwaukee Public Museum. In Wisconsin, at least, there appears to be no very clear-cut line between leaves 2–8 mm. wide (*G. pallida*) and leaves 2–3 mm. wide (*G. Fernaldii*), between anthers 1 mm. long (*G. pallida*) and anthers 0.2–0.5 mm. long (*G. Fernaldii*), between spikelets 4–7-flowered (*G. pallida*) and spikelets 3–5-flowered (*G. Fernaldii*), or between spikelets 6–7 mm. long (*G. pallida*) and spikelets 4–5 mm. long (*G. Fernaldii*). Furthermore, the degree of correlation of wider leaves with larger anthers and spikelets falls far short of

TABLE 1. *Glyceria pallida and var. Fernaldii in Wisconsin*

Locality	Width of widest leaf	Length of anther	Florets per spikelet	Length of spikelet
Merrill, Lincoln Co.	8.5 mm.	0.6 mm.	5–7	5.0–6.0 mm.
Neopit, Shawano Co.	5.0 mm.	0.8 mm.	4–5	4.0–5.0 mm.
Mosinee, Marathon Co.	5.0 mm.	0.8 mm.	4–5	4.0–5.5 mm.
Ashland, Ashland Co.	4.0 mm.	0.6 mm.	5–7	4.5–6.0 mm.
Laona, Forest Co.	3.5 mm.	1.8 mm.	3–4	3.5–5.5 mm.
Rainbow Rapids, Oneida Co.	3.5 mm.	0.2–0.4 mm.	3–4	3.5–4.0 mm.
Mellen, Ashland Co.	3.0 mm.	0.8 mm.	3–4	3.0–5.0 mm.
Ramadke, Clark Co.	3.0 mm.	0.4 mm.	4–6	5.0–5.5 mm.
Madeline Is., Ashland Co.	3.0 mm.	0.4 mm.	3–5	3.0–3.5 mm.
Price Co.	3.0 mm.	0.4 mm.	4–6	4.5–6.0 mm.
Rainbow Rapids, Oneida Co.	3.0 mm.	0.2 mm.	3–4	3.0–3.5 mm.
Moose River Dam, Douglas Co.	2.5 mm.	0.4 mm.	4–6	4.5–5.5 mm.
Ramadka, Clark Co.	2.0 mm.	0.4 mm.	4–6	4.5–5.5 mm.
Mather, Juneau Co.	2.0 mm.	0.4 mm.	3–4	3.5–4.5 mm.
Bruce, Rusk Co.	1.5 mm.	0.4 mm.	3–5	3.5–5.5 mm.
Grand View, Bayfield Co.	1.0 mm.	0.4–0.6 mm.	3–4	4.0–4.5 mm.
Phillips, Price Co.	1.0 mm.	0.4 mm.	3–4	3.5–4.0 mm.
Tomahawk, Lincoln Co.	1.0 mm.	0.4 mm.	3–4	3.0–4.0 mm.
Hayward, Sawyer Co.	1.0 mm.	0.4 mm.	3–5	3.5–5.0 mm.

TABLE 2. *Glyceria pallida and var. Fernaldii outside of Wisconsin*

Locality	Width of widest leaf	Length of anther	Florets per spikelet	Length of spikelet
New Bedford, Massachusetts	10.0 mm.	1.0 mm.	5–6	5.5–6.0 mm.
New Bedford, Massachusetts	8.0 mm.	1.0–1.4 mm.	6–7	6.0–7.0 mm.
New Bedford, Massachusetts	8.0 mm.	1.0 mm.	5–7	5.5–7.0 mm.
Harwich, Massachusetts	7.0 mm.	0.6 mm.	6–7	5.5–6.5 mm.
Noble Co., Indiana	5.5 mm.	1.0 mm.	3–4	3.5–4.5 mm.
Hanover, Massachusetts	5.0 mm.	0.8 mm.	5–6	5.0–6.5 mm.
New Bedford, Massachusetts	4.5 mm.	1.0–1.2 mm.	3–5	4.0–5.0 mm.
Barrington, New Hampshire	4.5 mm.	0.8 mm.	5–6	4.5–5.5 mm.
Durham, New Hampshire	4.5 mm.	0.6 mm.	3–5	4.5–5.0 mm.
Barrington, New Hampshire	4.0 mm.	0.8 mm.	4–5	5.0–5.5 mm.
Newfield, New Jersey	3.5 mm.	1.2 mm.	4–5	5.0–6.0 mm.
Orono, Maine	3.0 mm.	0.6 mm.	4–5	4.5–5.5 mm.
Amherst, Nova Scotia	3.0 mm.	0.4–0.6 mm.	5–6	4.5–5.0 mm.
Hartford, Connecticut	2.5 mm.	0.8–1.0 mm.	3–5	4.5–6.0 mm.
Temple, New Hampshire	2.5 mm.	0.4 mm.	3–5	3.5–5.0 mm.
Belgrade, Maine	2.5 mm.	0.4 mm.	2–5	3.0–5.0 mm.
Southport, Maine	2.0 mm.	0.4 mm.	4–5	3.5–5.0 mm.
St. Louis Co., Minnesota	2.0 mm.	0.4 mm.	3–5	4.0–6.0 mm.
Lake Co., Minnesota	2.0 mm.	0.4 mm.	5–6	4.0–6.0 mm.
Golden Lake, Ontario	2.0 mm.	0.4 mm.	3–4	3.5–4.5 mm.

demonstrating two clearly separable groups. Difference in lengths of anthers appears perhaps most definite among the characters listed by Dr. St. John, but in the plants of Wisconsin its correlation with other characters is not good.

From regions outside of Wisconsin, Dr. St. John undoubtedly had much more material at his disposal than has the present writer, but the characters of the 18 sheets tabulated in table 2 show only slightly better correlation of width of leaves with length of anthers and size of spikelets; they scarcely do more than suggest that var. *Fernaldii* is a smaller phase of *G. pallida*, perhaps recognizable as a northern variety.

HERBARIUM OF THE UNIVERSITY OF WISCONSIN
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STUDIES IN THE SAPOTACEAE—VI. MISCELLANEOUS NOTES

ARTHUR CRONQUIST

My revisions of several American groups of the *Sapotaceae*, under the auspices of the Chicle Development Company, are now concluded. During the course of that work various notes on other groups in the family have been accumulated. These are here presented, in order to make them available to future students of the family, and to make certain names available for use. An artificial key to the American genera of the family, as I understand them, is followed by notes and comments alphabetically arranged by genera. The citations of herbaria are the same as those used in previous papers of this series.

1. Sepals biseriate, mostly 3 plus 3, occasionally 4 plus 4, rarely 2 plus 2; corolla-lobes (except in a few species with 3 plus 3 sepals) with evident paired dorsal petaloid appendages. *Manilkara.*
1. Sepals not biseriate, or, if so, then 2 plus 2 (in *Pouteria*); corolla-lobes with lateral appendages (in *Dipholis* and *Bumelia*) or exappendiculate.
2. Staminodes present.
3. Stipules present, conspicuous. *Chromolucuma.*
3. Stipules wanting.
4. Corolla thick, fleshy, globose, with valvate lobes. *Sarcaulus.*
4. Corolla not especially thick, the lobes imbricate.
5. Seed-scar small, basilateral.
6. Corolla-lobes without lateral lobes; endosperm present. *Mastichodendron.*
6. Corolla-lobes with lateral lobes, except in a few species of *Bumelia.*
7. Endosperm present; ovary nearly always glabrous; unarmed. *Dipholis.*
7. Endosperm wanting; ovary usually hairy; usually more or less spiny. *Bumelia.*
5. Seed-scar long, lateral.
6. Endosperm present.
7. Leaves striate, alternate; filaments attached at the corolla-throat. *Micropholis.*
7. Leaves not striate.
8. Leaves conspicuously reticulate, alternate; filaments attached at the base of the corolla-tube. *Achrouteria.*
8. Leaves with arcuate-ascending primary lateral veins and numerous fine reticulate-parallel veins perpendicular to the midrib, opposite or subopposite; filaments attached at the corolla-throat. *Syzygiopsis.*
6. Endosperm wanting.
7. Leaves not glaucous; secondary lateral veins ordinarily evident below; staminodes generally well developed and regular. *Pouteria.*

7. Leaves more or less glaucous beneath, the secondary lateral veins obscure beneath; staminodes irregularly developed. species of *Oxythece.*

2. Staminodes absent.
3. Endosperm present. *Chrysophyllum.*
3. Endosperm wanting.
4. Locules 2–5, each containing one ovule; seed-scar long, lateral.
5. Distal portion of the filaments abruptly tenuous, reflexed in bud. *Pradosia.*
5. Distal portion of the filaments similar to the basal portion, not reflexed.
6. Stipules wanting; leaves usually more or less glaucous beneath, the secondary lateral veins obscure beneath. *Oxythece.*
6. Stipules usually present, though often deciduous; secondary lateral veins evident and raised beneath; leaves not glaucous, generally large and clustered at the ends of the twigs. *Ecclinusa.*
4. Locule 1, by failure of the partition; ovules 2; seed-scar relatively small, basilateral. *Diploön.*

Diploön Cronquist, gen. nov.

Tree; leaves alternate, weakly striate; flowers clustered in the axils or at recently defoliated nodes; sepals 5; petals 5, joined only at the base; stamens 5, epipetalous, opposite the corolla-lobes, the filaments basally much-thickened; staminodes none; ovary glabrous, unilocular by failure of the partition; ovules 2, basilaterally attached to a cushion-like basal placenta; fruit fleshy, single-seeded; seed with orbicular not very large basilateral scar; endosperm wanting.

Arbor foliis alternis tenuiter striatis; flores axillares, sepalis 5, petalis 5 ad basim connatis, staminibus 5 epipetalis corollae lobis oppositis, staminodiis nullis, ovario glabro uniloculare per septum defectum, ovulis 2; fructus monospermus semine exalbuminose area derasa moderate parva basilaterali orbiculari.

A single known species:

Diploön cuspidatum (Hoehne) Cronquist, comb. nov.

CHRYSOPHYLLUM CUSPIDATUM Hoehne, Ostenia 302. *pl. 8.* 1933.

An excellent description and plate of this species was provided by Hoehne. Careful examination of a number of flowers, including some kindly sent to me by Dr. Hoehne from the type tree, shows only vestiges of a partition between the ovules. All other American genera of the family, and so far as I am aware all of the old-world genera as well, have as many locules as ovules, and this feature has generally been considered to be a family character of the *Sapotaceae. Diploön* does not seem allied to any other family, however, and in my opinion the failure of the partition is not sufficient reason to exclude it from the *Sapotaceae.* Dr. Robert W. Hess, of the Yale University School of Forestry, has kindly examined a specimen for me, and tells me that there is nothing in the wood anatomy to preclude its being a member of *Chrysophyllum.* Aside from the structure of the ovary, *Diploön* differs from all other American *Sapotaceae* in the combination of

estaminodial flowers and relatively small basilateral seed-scar. It further differs from *Chrysophyllum* in the absence of endosperm. The glabrous ovary, while unusual in the family, is shared among the American *Sapotaceae* with *Mastichodendron* and *Dipholis*, both of which have staminodes and albuminous seeds. The conspicuously thickened filaments are not to my knowledge matched by any American member of the family, but this character may be of no more than specific importance.

The following specimens of *Diploon cuspidatum* have been examined; most of them are sterile.

Dusén s.n., Tacarahy, Paraná, December 12, 1914 (S); *Dusén 15319*, Serra do Prata, in silva primaeva, Paraná (S); *Hoehne 28358*, Jard. Bot. Sao Paulo (A, F, NY, US, Y); *Glaziou 9501*, entre Queimado et Rio d'Ouro, Rio de Janeiro (S); *Glaziou 9505*, near Rio de Janeiro (K), *19598*, near Rio de Janeiro (NY).

Mr. C. L. Gilly has kindly pointed out to me that the name *Manilkara emarginata* (L.) Britt. & Wils. (1926), which I adopted in my treatment of the North American species of *Manilkara* (Bull. Torrey Club **72**: 550–562. 1945), is a later homonym of *Manilkara emarginata* Lam (1925). The species I had in mind properly becomes *Manilkara jaimiqui* (Wright) Dubard, Ann. Col. Inst. Mars. III. **3**: 16. 1915. The following new combinations are necessary:

MANILKARA JAIMIQUI (Wright) Dubard subsp. **jaimiqui** (Wright) Cronquist, comb. nov. *Mimusops jaimiqui* Wright in Griseb. Cat. Pl. Cub. 64. 1866.

MANILKARA JAIMIQUI (Wright) Dubard subsp. **emarginata** (L.) Cronquist, comb. nov. *Sloanea emarginata* L. Sp. Pl. 512. 1753.

MANILKARA JAIMIQUI (Wright) Dubard subsp. **wrightiana** (Pierre) Cronquist, comb. nov. *Mimusops wrightiana* Pierre, Symb. Ant. **5**: 171. 1904.

MANILKARA JAIMIQUI (Wright) Dubard subsp. **haitensis** (Cronquist) Cronquist, comb. nov. *Manilkara emarginata* subsp. *haitensis* Cronquist, Bull Torrey Club **72**: 558. 1945.

In my treatment of the North American *Sapotaceae* (Lloydia, in press), I included the single species of *Oxythece* in *Pouteria*, following Eyma's reasoning that the presence of irregularly developed staminodes in some species (including the North American one) invalidated the distinction between the two genera. I now feel that, although the technical character does break down, *Oxythece* is better treated as a distinct genus. It apparently forms a natural, readily recognizable group, not too difficult of definition, and may be nearly as closely related to *Pradosia* as to *Pouteria*.

Oxythece pallida (Gaertn. f.) Cronquist, comb. nov.
Lucuma pallida Gaertn. f. Fruct. **3**: 130. *pl. 204*. 1807.
Oxythece hahnianum Pierre ex Duss. Ann. Inst. Col. Mars. **3**: 387. 1897.
Oxythece fabrilis Pierre, Symb. Ant. **5**: 160. 1904.
Pouteria pallida Baehni, Candollea **9**: 352. 1942.

It is interesting to note that Baehni is so impressed by the importance of the development of staminodes, as opposed to endosperm, as the distinction between *Pouteria* and *Chrysophyllum* (in his greatly extended definition of each) that he dismembers this species and refers the estaminodial extreme (*Oxythece fabrilis*) to "*Chrysophyllum* sp."

Oxythece schomburgkianum (Miq.) Cronquist, comb. nov.
Myrsine schomburgkiana Miq. in Mart. Fl. Bras. **10**: 315. 1856.
Chrysophyllum oleaefolium Spruce ex Miq. in Mart. Fl. Bras. **7**: 101. 1863.

This species has the leaves merely glabrous rather than glaucous beneath, with the secondary lateral veins a little more evident than is usual for the group, but is otherwise very similar to *Oxythece*, and does not seem to belong anywhere else. Short of reviving the ill-defined monotypic segregate *Elaeoluma* for it, the species must be referred to *Oxythece*.

Oxythece ferreirii Cronquist, sp. nov. Twigs coarse, glabrous; leaves opposite or offset, glabrous, the blade elliptic, blunt, 8–15 cm. long, 4–6.5 cm. wide, the petioles stout, about 1–1.7 cm. long; primary lateral veins arcuate, impressed above, slightly impressed and loosely reticulate beneath; secondary lateral veins obscure above, slightly impressed and loosely reticulate beneath; flowers clustered in the axils, the pedicels glabrous, about 4–5 mm. long; sepals glabrous, rounded, elliptic or ovate, about 3.5–6 mm. long; corolla glabrous, about 4–6 mm. long, the tube very short; staminodes none; filaments straight, attached at the level of the sinuses; anthers about 2 mm. long; ovary closely appressed-hairy, 3-locular; style glabrous, 2 mm. long; fruit unknown.

Arbor, ramulis crassis glabris, foliis glabris ellipticis obtusis 8–15 cm. longis 4–6.5 cm. latis, petiolo 1–1.7 cm. longo, venis primariis arcuatis supra impressis infra vix elevatis, venulis supra obscuris infra tenuiter impressis laxe reticulatis; flores axillares pedicellis glabris 4–5 mm. longis, sepalis glabris 3.5–6 mm. longis, corolla glabra 4–6 mm. longa tubo brevissimo, staminodiis nullis, filamentis fauce affixis antheris 2 mm. longis, ovario 3-loculare, stylo glabro 2 mm. longo; fructus ignotus.

TYPE: *Ferreira 516*, Brazil (K).

Additional specimen: PERU: Loreto: *Williams 3669*, Iquitos (F).

This species is obviously related to *O. schomburgkianum*, but differs in its broader leaves, longer petioles, and slightly larger flowers.

Pouteria subsessilifolia Cronquist, sp. nov. Shrub about 3 m. high, with thick scanty latex; young twigs rather coarse, finely and rather loosely hairy; leaves firm, elliptic-oblong, obtuse or rounded at the apex, rounded or subcordate at the base, about 3.5–6.5 cm. long and 16–37 mm. wide, pubescent on both sides with short-stalked two-forked rufous hairs, soon becoming glabrous above, only in age if at all glabrate below, but the hairs eventually fading; primary lateral veins about 10–18 pair, not very prominent; secondary lateral veins obscure; petioles short and stout, about 2–4 mm. long; flowers about 5–15 in axillary clusters which may develop an axis as much as 15 mm. long; pedicels rufous-hairy, about 6–9 mm. long; sepals 5, hairy like the pedicels, about 1.8–3.5 mm. long; corolla about 3.2–

3.8 mm. long, sometimes scarcely exceeding the sepals, the 5 lobes rounded, slightly auriculate, a little longer than the tube; filaments short, attached about at the level of the sinuses; anthers introrse, 1.0–1.3 mm. long; staminodes irregularly developed, about 0.2–0.6 mm. long, or some of them apparently wanting; ovary 2-locular, with laterally attached ovules; style glabrous, about 1.5 mm. long; fruit unknown.

Frutex 3 m. alta, foliis elliptico-oblongis subsessilibus basi rotundatis vel subcordatis, 3.5–6.5 cm. longis 16–37 mm. latis, utrinque molliter pubescentibus supra glabratis, venis primariis ca. 10–18-jugis vix prominentibus, venulis secondariis obscuris; flores axillares 6–9 mm. longe pedicellati, sepalibus 5 rufo-pubescentibus 1.8–3.5 mm. longis, corolla ca. 3.2–3.8 mm. longa 5-lobata lobis tubi paullo superantibus, filamentis brevibus fauce affixis, antheris introrsis, staminodiis irregulariter productis ca. 0.2–0.6 mm. longis, ovario 2-loculare stylo glabro 1.5 mm. longo; fructus ignotus.

TYPE: *Froes 1023*, Andarahy, Serra do Cornelio, basin of River Paraguassu, Bahia, Brazil, October 13, 1942 (A); isotype in herb. B. A. Krukoff.

Local name: Massaranduba.

The affinities of this species are uncertain. Its 5-merous flowers and 2-celled ovary exclude it from any of the sections as defined by Baehni. In his artificial key to the species it becomes lost among several strictly old-world species which are referred by other authors to *Planchonella*. Since several species of *Pouteria* are known to have both 4-merous and 5-merous flowers, and there is a group of species with 4-merous flowers and 2-celled ovaries, its affinities may well lie with these, the section *Discoluma* as defined by Baehni. It is interesting to note that several of the species included by Baehni in this section, which he clearly defines on page 219 of his treatment to include only species with 4-merous flowers, are described by him as having 5 sepals and 5 corolla-lobes. Within this section, the broadly rounded to subcordate bases of the subsessile leaves distinguish *P. subsessilifolia*, and further vegetative and floral difference are seen when it is compared with any one of the species.

Pouteria trilocularis Cronquist, sp. nov. Tree about 25 m. high, buttressed at the base, with thick scanty latex; young twigs closely and obscurely sericeous, soon glabrate; leaves rather narrowly elliptic-obovate or merely elliptic, acuminate to occasionally rounded or obtuse at the apex, narrowed to the base, about 10–28 cm. long and 3–11 cm. wide, glabrous; primary lateral veins about 7–12 pair; secondary lateral veins tending to become perpendicular to the primary ones, toward the margin, but irregular and not much more prominent than the conspicuous and not very fine reticulum; petioles about 1–2.5 cm. long; flowers fairly numerous in axillary clusters or at recently defoliated nodes, the pedicels sericeous, about 4–8 mm. long; sepals 5, sericeous, firm, ovate or deltoid-ovate, acute or acuminate, about 3.4–4.0 mm. long; corolla firm, glabrous, tubular, scarcely exceeding the sepals, about 3.6–4.7 mm. long, the lobes about as long as the tube; filaments short and thick, only about 0.5 mm. long, inflexed, attached at or near the level of the sinuses, often without anthers; anthers about 0.7–1.2 mm. long; staminodes glabrous, inflexed, ovate, acuminate, about 0.8–1.1

mm. long; ovary depressed, almost disciform, closely hairy, obscurely about 6 lobed, 3-locular; style glabrous, nearly 2 mm. long; stigma-lobes 3; young fruits show enlargement of one locule at the expense of the others, with seeds attached along a narrow line for their whole length, indicating that the mature fruit will probably be 1-seeded, and the seed have a long linear scar; mature fruit not seen, but reputedly 3 cm. thick, yellow, and edible.

Arbor 25 m. alta, ramulis juventutis obscure sericeis mox glabratis, foliis glabris ellipticis vel saepius elliptico-obovatis ca. 10–28 cm. longis 3–11 cm. latis, venis primariis 7–12-jugis, petiolis ca. 1–2.5 cm. longis; flores fasciculati pedicellis 4–8 mm. longis, sepalibus sericeis acutis 3.4–4.0 mm. longis, corolla glabra 3.6–4.7 mm. longa, lobis tubo subaequalibus, filamentis crassis 0.5 mm. longis fauce affixis saepe anantheris, antheris 0.7–1.2 mm. longis, staminodiis inflexis acuminatis 0.8–1.1 mm. longis, ovario depresso 3-loculare, stylo glabro vix 2 mm. longo; fructus edulis aureus 3 cm. crassus traditum est.

TYPE: *A. Moysey Adams 2070/w11*, terra firma, Macapa, Baco de Acre, basin of Rio Purus, Acre Territory, Brazil, April 26, 1944 (NY).

Local name: Abiurana.

Three other collections, all in young fruit, are known from Acre Territory, near the mouth of the Rio Macauhan, a tributary of the Rio Vaco, in the basin of the Rio Purus. These are *Krukoff 5283, 5338,* and *5531.* They were distributed as *Sideroxylon bolivianum,* which is a synonym of *Chrysophyllum gonocarpum.*

This species is related to *P. inflexa* (A. C. Smith) Baehni, from northwestern Matto Grosso, and *P. obscura* (Huber) Baehni, from 1000 miles away in Para. It agrees with both of these in its 5-merous flowers, corolla-lobes about as long as the tube, filaments attached at the corolla-throat, and trilocular ovary. The three species may be distinguished by the following key:

1. Ovary ovoid or subglobose, its style about 4 mm. long; staminodes erect; filaments about as long as the anthers; leaves rufous-tomentose along the midrib beneath; sepals and young twigs rufous-tomentulose; pedicels about 2–3 mm. long; Para. *P. obscura.*
1. Ovary depressed, its style about 2 mm. long or less; staminodes inflexed; filaments distinctly shorter than the anthers, only about 0.5 mm. long; leaves glabrous; sepals and young twigs merely sericeous; pedicels 4–40 mm. long; Acre Territory and northwestern Matto Grosso.
   2. Corolla glabrous; pedicels about 4–8 mm. long; flowers more than 5 in a cluster; staminodes acuminate; leaves about 10–28 cm. long, on petioles 1–2.5 cm. long. *P. trilocularis.*
   2. Corolla sericeous; pedicels about 25–40 mm. long; flowers about 2–5 in a cluster; staminodes obtuse; leaves about 8–12 cm. long, on petioles 7–12 mm. long. *P. inflexa.*

Pradosia mutisii Cronquist, sp. nov. Similar to *P. glycyphloea,* of southeastern Brazil, in its small (up to 12.5 × 5 cm.), alternate leaves with long (1–3 cm.) slender petioles, and in its small subsessile flowers; differing in the more numerous primary lateral veins of the leaves (about 15–25 pair), in the evident close reticulum of the secondary veins, in the glabrous sepals, and in the short conic style only about 0.5 mm. long; fruit unknown.

A *P. glycyphloeae* differt venis numerosioribus (15–25-jugis), venulis dense et perspicue reticulatis, sepalis glabris, stylo brevi ca. 0.5 mm. longo.

TYPE: *Mutis 4004*, Colombia (US). There are three other sheets at US, bearing the numbers *192*, *1020*, and *2324*, but on each of these has been penciled = 4004, and all four sheets look enough alike to have come from the same collection.

Syzygiopsis sericea Cronquist, sp. nov. Tree 10–15 m. tall, with an irregular crown; leaves opposite or slightly offset, elliptic, 5–11.5 cm. long, 2.5–5.5 cm. wide, obtuse or with a short blunt acumen, glabrous and shining above, rufous-sericeous beneath, glabrate or subglabrate in age; primary lateral veins about 6–8 pair, arcuate, strongly ascending, sharply raised beneath; secondary lateral veins numerous, fine, parallel, nearly perpendicular to the midrib, hidden beneath the pubescence; petioles flattened, about 6–10 mm. long; flowers small, the persistent reniform sepals less than 2 mm. long; fruit ellipsoid-globose, bitter, about 2 cm. long; seed solitary, compressed, with a long narrow lateral scar; endosperm present; embryo with thin cotyledons and well-developed caudicle.

Arbor 10–15 m. alta, foliis oppositis vel suboppositis ellipticis 5–11.5 cm. longis 2.5–5.5 cm. latis obtusis vel obtuse breviterque acuminatis supra glabris infra rufo-sericeis in extremis plus minusve glabratis, venis lateralibus primariis arcuato-ascendentibus 6–8-jugis, venulis numerosis tenuissimis reticulato-parallelis ad costam perpendicularibus, petiolis 6–10 mm. longis; flores parvi sepalis vix 2 mm. longis; fructus ellipsoideo-globosus 2 cm. longus; semen solitarium area derasa lineari laterali albumine recondito.

TYPE: *Llewelyn Williams 15074*, San Antonio, Rio Orinoco, Venezuela, 121 meters, April 27, 1942 (US).

In the absence of flowers, the generic position of this species cannot be determined with absolute certainty, but the leaf-venation (especially the numerous fine parallel secondary veins extending at right angles to the midrib) is so similar to that of *Syzygiopsis appositifolia* that I am convinced the two are congeneric. *Syzygiopsis* is the only endospermous American genus of the family known to have opposite leaves, but too much stress should not be placed on this feature, since several non-endospermous genera must perforce include both opposite-leaved and alternate-leaved plants.

Syzygiopsis sericea differs from *S. oppositifolia* in its shorter, broader, blunter leaves, that have fewer primary lateral veins and are conspicuously sericeous beneath, the leaves of *S. oppositifolia* being glabrous.

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TORREYA

PROCEEDINGS OF THE CLUB

Minutes of the Special Meeting of January 8, 1946. The special meeting of the Torrey Botanical Club on Tuesday, January 8, 1946, marking the closing of the Fiftieth Anniversary celebration of the founding of The New York Botanical Garden began with a luncheon at 1:00 p.m. in the Member's Room of the The New York Botanical Garden with 60 people present. After a delightful luncheon and social hour, the scientific program was opened at 2:00 p.m. by President Seaver. Seventy-five people attended this. Dr. Robbins, Director of The New York Botanical Garden, spoke a few words about the history and relationship of the two organizations.

The following addresses were presented:

Professor Lauren C. Petry—"The Place of Botany in General Education."

General education has been defined as "education for an informed responsible life in our society." The teaching of the sciences in the colleges and universities has been more effective in informing students than it has been in increasing their awareness of their responsibilities or their tendency to act in accordance with them. It is proposed to organize the subject-matter of botany courses on the basis of the fundamental concepts and generalizations of the science, and to adopt teaching methods designed to develop in students habits of analysis, criticism, theory formulation, verification, and generalization. It is further proposed that teachers of science at the college level should discuss in detail the practical social, economic, and political problems which science is creating and should indicate the methods to be employed in seeking solutions.

Professor Jacob R. Schramm—"The Object of a Course in Botany for College Students."

Professor Edmund W. Sinnott—"Botany in the Next Half Century."

At a time when we celebrate the close of half a century for an institution like The New York Botanical Garden, it is perhaps worth while to look ahead to the next few decades and try to see the development which botanical science may be expected to undergo in the future.

The great advances in the physical sciences will certainly have their repercussions in botany. New techniques—"tagged" atoms, radiations of many sorts, the electron microscope and others—will make it possible to attack problems which were heretofore insoluble. Among these are the physical and chemical structure of living substance, about which we may expect to learn a great deal in the future. The activities that go on in living systems can also be analyzed far more fully than before. The advances in biochemistry, particularly, will make it possible to follow in more complete detail the various stages in such metabolic reactions as are concerned in respiration and photosynthesis. Growth and development will be an active spot. The general problems of morphogenesis—the controlled development of living things—which is perhaps the central and ultimate problem of biology may be expected to yield at least in part to new methods of attack. Evolution, the problems of which were thought to be largely solved a generation ago, will certainly be actively studied in the future with the techniques of genetics and cytology. Taxonomy is already being revitalized by such research. The problems of the past history of the plant kingdom are by no means solved and much remains to be done here. Indeed, the plant population of the earth is only imperfectly known even today and we may expect much from a fuller exploration of all parts of the world.

Many new practical applications will result from this advanced knowledge in the plant sciences. In agriculture the continued application of knowledge of

genetics will undoubtedly produce improved types of plants and better control of disease. The tremendous increase in our chemical knowledge by which so many products heretofore made only by plants can now be synthesized will have its effect upon agriculture. However, even with these advances in chemistry the fundamental syntheses of carbohydrates and amino acids are still made only by plants. It may well be that in the future agricultural crops will be grown primarily for total carbohydrate or protein yield rather than for specific products. The development of tropical agriculture should be important in this connection. The sea produces an enormous amount of plant material, only a tiny fraction of which is now utilized.

Despite the development of atomic power much of the energy needed by our industrial civilization will still come from plant products. As coal and petroleum become scarcer, we shall have to depend on photosynthesis by living plants for this purpose. Techniques are already known by which much of the photosynthetic activity of the globe can be used for the production of fuel.

In medicine the discovery of penicillin shows how important plant syntheses can be for the healing of disease. Many new antibiotics are now being developed from the lower plants and botany is again assuming its ancient role as a source of medicinal materials.

Predicition is rash and many unexpected events may change the future of our science, but the next fifty years will certainly be a time of adventure and excitement for botanists of every sort.

After discussion of these papers, the group was adjourned at 3:45 p.m.

Respectfully submitted,
FRANCES E. WYNNE,
Recording Secretary

Minutes of the Annual Meeting of January 8, 1946. The annual dinner meeting was held on the evening of January 8th at the Men's Faculty Club of Columbia University. There were 94 members and friends present. The business meeting was called to order by the retiring president, Dr. Seaver, at 7:30 p.m. The minutes were approved as read.

Two persons were elected to membership in the club.

The following officers and chairmen of standing committees made reports:

Dr. Simpson, *Corresponding Secretary*
Dr. Wynne, *Recording Secretary*
Dr. Fulling, *Treasurer*
Dr. Rickett, *Editor*
Dr. Clum, *Editor of Torreya*
Dr. Small, *Chairman of the Field Committee*
Dr. Karling, *Historian*
Dr. Dodge, *Delegate to the N. Y. Academy of Sciences.*

Dr. Robbins spoke a few words on the relationship between the New York Botanical Garden and the Torrey Botanical Club and the club's part in founding the Garden.

Dr. Seaver then introduced the guest speaker of the evening, Dr. Joseph Brandt, President of Henry Holt & Co., who gave a very interesting and stimulating paper, "Science Writing and Politics."

Dr. Seaver then announced the election of officers as follows:

President—Dr. P. W. Zimmerman
1st Vice President—Dr. John A. Small
2nd Vice President—Dr. Henry K. Svenson
Corresponding Secretary—Dr. Jennie L. S. Simpson
Recording Secretary—Dr. Frances E. Wynne
Treasurer—Dr. E. H. Fulling

Editor—Dr. Harold W. Rickett
Bibliographer—Mrs. Lazella Schwarten
Business Manager—Dr. Anne Hanson
Delegate to the Council of the N. Y. Acad. of Sciences—Dr. Bernard O. Dodge

Members of the Council

Dr. George S. Avery
Dr. W. H. Camp
Dr. Arthur H. Graves
Dr. George H. Shull

Representative on the Council of A.A.A.S.

Dr. George S. Avery
Dr. Michael Levine

Representative on the Board of Managers of the New York Botanical Garden

Dr. Henry A. Gleason

Dr. Karling moved that the officers elected by vote be approved. The motion was seconded and passed.

The meeting was then turned over to the incoming President, Dr. Zimmerman, who spoke a few words of greeting to the club.

The meeting was adjourned at 8:30 p.m. to permit informal discussion and conversation with friends.

Respectfully submitted,
FRANCES E. WYNNE,
Recording Secretary

Minutes of the Meeting of January 16, 1946. The regular meeting of the Torrey Botanical Club was called to order by President P. W. Zimmerman at The New York Botanical Garden with 35 members and friends present. Minutes of the annual meeting and announcements of new officers were read and approved.

The resignation of Dr. Frances E. Wynne as Recording Secretary was accepted, and Dr. E. E. Naylor nominated and elected as acting Recording Secretary.

President Zimmerman named three members to function as a committee for the Cumulative Index of the *Bulletin* as follows: Dr. Seaver, Chairman, Dr. Rickett, and Dr. Karling.

It was moved and seconded that reprints of the section of the *Bulletin*, formerly *Torreya*, be sent to associate members for a period of one year at a total cost to the club of not more than $100.

President Zimmerman asked for suggestions for a suitable chairman of the Field Committee to replace Dr. Small. Some discussion followed. Mr. R. Platt and Dr. Svenson were suggested, but no action was taken.

The scientific portion of the program consisted of a very interesting and informal talk by Dr. W. H. Camp on "Plant Hunting in Ecuador."

The meeting was adjourned at 4: 30 p.m. for tea and general discussion.

Respectfully submitted,
FRANCES E. WYNNE,
Recording Secretary

Minutes of the Meeting of February 5, 1946. The regular meeting of the Torrey Botanical Club was called to order by President Zimmerman at 8:15 p.m. at Columbia University. Minutes of the meeting of January 16th were approved as read.

President Zimmerman announced that Mr. Ajello of Columbia University had been suggested as a candidate for the office of Recording Secretary for the Club. Mr. Ajello was nominated and elected without discussion.

Two new members, already passed at the Council meeting were elected to associate membership.

The only other business was the appointment by the President of Dr. Cornelia L. Carey and Miss Honor M. Hollinghurst to fill vacancies in the council.

The scientific program of the evening was a paper presented by Dr. George M. Hocking on "The Pharmaceutical Botany of Peppermint." The speaker's abstract follows:

> First, pharmaceutical botany (or medical botany) was defined as paralleling in a general way the various fields of general botany, but the plants studied are of medicinal interest: the chief emphasis being put of course on practical aspects, as far as this is possible. Discussion then followed on the importance of Peppermint, as reflected in its wide usage, cultivation and inclusion in pharmacopoeias. Its nomenclature was briefly discussed, together with what is known of its hybrid origin, discovery and spread from England to the Continent and then to America. Areas of cultivation in this country, mode of propagation and tillage, methods of harvesting and distillation were considered.
>
> The reputedly largest mint farm at Mentha, Michigan, was briefly described. The importance of the volatile oil and its chief component, menthol, were discussed. The uses of the plant were illustrated and its importance technically as well as in foods, medicines and masticatories was indicated. All parts of the plant are used, even the spent herb coming from the still.
>
> Three useful means of identification of the leaf (etc.) by histological methods were detailed: (a) leaf vein islet counts, (b) trichome characteristics, and (c) palisade cell ratios. Of these, the first and second are frequently adequate in fixing identity of a powdered leaf material, whereas the third method is used mainly for confirmation.
>
> The author has carried out determinations for various species of *Mentha* with both leaf vein islet counts and palisade ratios. In this work, he found that the value of vein islet counts for plants growing outside during the summer months showed a high degree of correlation within the species, but that leaves developed in the winter (in greenhouse) showed lower values in plants from identical stocks.
>
> Trichomes of mechanical type are of considerable help in identification of species, as was illustrated, whereas glandular hairs are considered of very little value on account of the similarity of these structures in various species of *Mentha* and indeed throughout most Labiates.

Twenty-six members were present. The meeting adjourned at 9:15 p.m. for tea served by members of the Botany Department of Columbia University.

Respectfully submitted,
E. E. Naylor,
Acting Recording Secretary

Minutes of the Meeting of February 20, 1946. The regular meeting of the Torrey Botanical Club was called to order by President Zimmerman at 3:30 p.m. at The New York Botanical Garden. Twenty-five members were present. Minutes of the meeting of February 5th were approved as read.

There was no new or old business to transact and accordingly the meeting was turned over to Dr. Fredda D. Reed, who presented a talk on "Carboniferous Plants and Some Problems of Evolution."

The meeting adjourned at 4:45 p.m. for tea served by members of The New York Botanical Garden.

Respectfully submitted,
Libero Ajello,
Recording Secretary

Minutes of the Meeting of March 5, 1946. The regular meeting of the Torrey Botanical Club was called to order by Dr. Zimmerman at Hunter College at 8:15 p.m. with 150 members and guests present.

The minutes of the meeting of February 20th were approved as read.

The recording secretary read the following motion which was approved unanimously: "That the Club go on record as favoring an arrangement with the Society of American Plant Taxonomists to furnish this Society at cost price reprints of the 'Index to American Botanical Literature' from the *Bulletin* for distribution to the members of the Society in place of the 'Taxonomic Index.'"

Dr. J. S. Karling presented a report on the Biological Institute which is to be organized at the coming St. Louis meetings. This institute will fill the need for a close-knit biological organization which will be able to represent biologists in national and other affairs.

There was no further business, and so the meeting was turned over to Mr. Rutherford Platt who presented a most stimulating lecture on "Art Forms in Nature" illustrated with excellent kodachromes.

The meeting adjourned at 9:20 p.m. and tea was served by the Hunter Faculty.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

Minutes of the Meeting of March 20, 1946. The regular meeting of the Torrey Botanical Club was called to order by Dr. Zimmerman at Fordham University at 4:00 p.m. with 57 members and guests present.

The minutes of the March 5th meeting were approved as read.

Dr. Simpson announced that there is to be an additional meeting of the Torrey Botanical Club on the evening of Monday, April 15th, at Columbia University. Dr. J. Van Overbeek will address the Society at that time.

There was no further business and the meeting was turned over to Dr. C. A. Berger who presented a paper on "Naturally Occurring Tetraploidy in the Development of *Allium*." The speaker's abstract follows:

> Polyploid mitotic divisions were found to occur in the large cortex cells of the cotyledon of all young seedlings of *Allium cepa*. They were very abundant in seedlings approximately 15 mm. long, where they were found throughout the cotyledon. In seedlings about 20 mm. long these divisions were concentrated at the base of the cotyledon. In seedlings longer than 20 mm. polyploid divisions were very scarce.
>
> In the long resting stage preceding the division, a double reduplication of the chromosomes takes place. As a result from earliest prophase to metaphase the chromosomes consist of four chromatids held together by a single undivided spindle attachment region (tetra-chromosomes). At metaphase two successive divisions of the spindle attachment region occur. After the first division the tetraploid number of metaphase chromosomes can be counted and the chromosomes are arranged in pairs. The second division of the spindle attachment region results in the separation of chromatids, which then undergo normal anaphase movement. Two tetraploid cells result. These polyploid cells apparently undergo only one mitotic division, since only metaphases with paired chromosomes were found.
>
> This is one of the few cases in which the role of polyploidy in normal development is shown by the direct evidence of polyploid mitotic divisions.

The meeting adjourned at 5:00 p.m.

Tea and coffee were served prior to the meeting by members of the Fordham Faculty.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

Minutes of the Meeting of April 2, 1946. The regular evening meeting of the Torrey Botanical Club was called to order by Dr. Zimmerman at Columbia University at 8:30 p.m. with 26 members and guests present.

The minutes of the March 20th meeting were approved as read.

Dr. Zimmerman announced that Dr. J. Van Overbeek is to address the club at a special meeting at Columbia University on April 15th.

The speaker of the evening, Dr. Robbins, was not able to attend and the meeting was turned over to Dr. Karling and Dr. Zimmerman who informally discussed the meetings of the A.A.A.S. held recently in St. Louis.

The meeting adjourned at 9:15 p.m. Tea and cakes were served by members of the Columbia Faculty.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

Minutes of the Special Meeting of April 15, 1946. The special meeting of the Torrey Botanical Club was called to order by Dr. J. A. Small at 8:15 p.m. at Columbia University with 34 members and guests present.

The reading of the minutes of the previous meeting was omitted and Dr. Small introduced the speaker of the evening, Dr. J. van Overbeek, head of the Department of Plant Physiology of the Institute of Tropical Agriculture, Mayagüez, Puerto Rico. The speaker's abstract follows. This was prepared by Dr. Marie E. Conklin at the speaker's request.

Emphasizing that the plant research program at Mayagüez is being directed toward solving problems of both a purely scientific and a practical nature, Dr. van Overbeek proceeded to cite examples from each of these categories of research which is currently in progress at the Institute. Investigation of both chemical and biological aspects of the relation of hormones to the growth, rooting, flowering and fruiting of tropical plants and the application of the results of these investigations to tropical agricultural has been the main objective of this research group.

Fundamental research on the physiological function of leaves in the process of root formation has been conducted on cuttings of *Hibiscus* and other plants. It was found that cuttings, from which leaves had been removed, rarely rooted, even with the addition of indole butyric acid. A combination of indole butyric acid and nitrogenous compounds such as ammonium sulphate or arginine in the presence of sucrose, produced excellent root formation comparable to that of the cuttings with leaves attached and indole butyric acid added. These data clarified the relation of hormones and plant nutrients in the process of root formation. Cuttings of *Cacao* and *Hevea*, which are extremely difficult to root, were made to root by applying the method of submerging in water immediately after cutting to avoid plugging the vascular system with the plant's mucoid exudate.

Pineapples, it was found, could be stimulated to bear flower and fruit, if treated with 0.001% naphthalene acetic acid (or 2–4D), applied to the center of the plant. The exact weight and size of fruit can be predicted, for there is a definite correlation between the number of leaves on the plant at the time of treatment, and the resulting fruit. By using for treatment a variety of pineapple which under natural conditions flowers with difficulty, complete crop control is possible. Thus for a cost of one dollar, over 50 acres of pineapples can be treated, and both the time of harvest and the size of the fruit are controlled. The possibility of hormonal control of flowering in other plants is under investigation.

Use of hormones, or hormone analogs for weed control, particularly in the sugar cane fields, is proving very successful in Puerto Rico. Hand hoeing of sugar cane fields has been the usual procedure, at a cost of about $40.00 per acre. By spraying with 0.05 to 0.1% 2–4D, *Commelina*, *Cyperus*, and other weeds are effectively destroyed without damage to the cane, at a cost of $4.00 or less per acre. Weed eradication in cane fields is very important to insure maximum yield of

sugar by permitting cuttings close to the ground. *Ipomoea* can also be successfully controlled in this way in coffee plantations, where it smothers the trees.

Following his presentation of scientific data, Dr. van Overbeek showed a series of beautiful kodachrome slides from the large collection he has made while in the tropics. They were of unusual interest because they showed the natural beauty of Puerto Rico, while serving to illustrate the experimental work which is being conducted on the native economic crops of sugar cane, pineapple, coffee, vanilla and mango.

The meeting adjourned at 9:15 p.m.

Tea and cakes were served by members of the Columbia faculty.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

Minutes of the Meeting of April 17, 1946. The regular afternoon meeting of the Torrey Botanical Club was called to order by Dr. Zimmerman at the American Museum of Natural History at 3:45 p.m. with 9 members and guests present.

The minutes of the April 2nd meeting were approved as read.

Dr. Zimmerman noted that the change in location of the day's meeting was an attempt to improve afternoon attendance by selecting a more centrally located meeting place.

There was no further business and the meeting was turned over to Dr. Svenson who spoke on the "Relation of the Galapagos Flora to That of the South American Mainland."

The meeting adjourned at 5:00 p.m.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

Minutes of the Meeting of May 1, 1946. The regular evening meeting of the Torrey Botanical Club was called to order by Dr. Zimmerman at Columbia University at 8:00 p.m. with 37 members and guests present.

The minutes of the April 17th meeting were approved as read.

There was no business to transact and the meeting was turned over to Dr. W. J. Robbins, who spoke on some of his recent research on *Phycomyces Blakesleeanus* and some of the Dermatophytes.

The meeting adjourned at 9:30 p.m. Tea and cakes were served by members of the Columbia faculty.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

NEWS NOTES

Books and Scientific Periodicals Needed. During the war, the libraries of half the world were destroyed in the fires of battle and in the fires of hate and fanaticism. Where they were spared physical damage, they were impoverished by isolation. There is an urgent need—now—for the printed materials which are basic to the reconstruction of devastated areas and which can help to remove the intellectual blackout of Europe and the Orient.

There is need for a pooling of resources, for coordinated action, in order that the devastated libraries of the world may be restocked as far as possible with needed American publications. The American Book Center for War Devastated Libraries, Inc. has come into being to meet this need. It is collecting and shipping abroad scholarly books and periodicals which will be useful in research and necessary in the physical, economic, social and industrial rehabilitation and reconstruction of Europe and the Far East.

Since shipping facilities are precious and demand that all materials be carefully selected, emphasis is placed upon publications issued during the past decade, upon scholarly books which are important contributions to their fields, upon periodicals (even incomplete volumes) of significance, upon fiction and non-fiction of distinction. All subjects—history, the social sciences, music, fine arts, literature, and especially the sciences and technologies —are wanted.

The following materials are not needed: textbooks, outdated monographs, recreational reading, books for children and young people, light fiction, materials of purely local interest, popular magazines such as *Time, Life, National Geographic,* etc., popular non-fiction of little enduring significance such as Gunther's *Inside Europe,* Haliburton's *Royal Road to Romance,* etc. Only carefully selected federal and local documents are needed, and donors are requested to write directly to the Center with regard to specific documents.

The Center cannot purchase books and periodicals; it must depend upon gifts from individuals, institutions, and organizations. Each state will be organized to participate in the program through the leadership of a state chairman. Other chairmen will organize interest in the principal subject fields. Cooperation with these leaders or direct individual contributions are welcome. When possible, periodicals should be tied together by volume, and missing issues should be noted on incomplete volumes. All shipments should be sent prepaid, via the cheapest means of transportation, to The American Book Center, c/o The Library of Congress, Washington 25, D. C. Although the Center hopes that donors will assume the costs of transportation of their materials to Washington, when this is not possible reimbursement will be made upon notification by card or letter of the amount due. The Center cannot accept material which is sent collect. Reimbursement cannot be made for packing or other charges beyond actual transportation.

The National Tribute Grove. Recently we received a reprint from the *Congressional Record* of some remarks made by Representative Clarence F. Lea about a year ago concerning this tract of redwood forest which is being preserved in honor of the men and women who have served in the armed forces of the United States in World War II. It is established as a public park by the action of the California State Park Commission with the cooperation of the Save-the-Redwoods League, the Garden Club of America, and other organizations. The object is to preserve this grove to symbolize "the eternal gratitude of the Nation eternally expressed." "Citizens throughout the United States are joining with the California park Authorities in assuring the acquisition and preservation of these mighty trees. The forest selected is that of the Mill Creek redwoods, near Cresent City, Calif., and will be a part of the California State park system.

"When completed by private contributions and State funds, the National Tribute Grove will contain more than 5,000 acres of some of the finest redwood forest. Some 1,400 acres, at a cost $260,000, are yet to be acquired under a purchase agreement with the timber owners. Not only will the grove be a symbol of gratitude to all who served in the armed forces, but there will also be opportunity for contributors to record the names of specific members of these forces in The Golden Book, which is to be a permanent volume, enshrined both in Washington, D. C., and in California.

"A national committee of sponsors has been formed, with Hon. Joseph C. Grew, of Washington, D. C., as chairman, and Dr. Robert G. Sproul, president of the University of California, as treasurer."

Mr. Lea goes on to say that the trees of the National Tribute Grove are coast redwoods, *Sequoia sempervirens,* the forests of which are being preserved by a system of State parks and the Muir Woods National Monument near San Francisco, which was a gift to the Nation by the late William Kent. Many of these trees are around 350 feet high and 12 to 17 feet in diameter, measured 5 feet from the ground, and some are known to be more than 2,000 years old. *Sequoia gigantea,* a species of older and more massive trees of the Sierra, has been preserved by the Federal Government in Sequoia, Kings Canyon, and Yosemite National Parks.

"The Naturalists' Directory," edited by Herman E. Cassino, contains the names, addresses, and special subjects of study, of professional and amateur naturalists through-

out the world. This handy directory has been published regularly for 60 years. The current edition is expected in September.

BOOK REVIEW

Turtox Microscopy Booklet. By A. C. Lonert. 1–43. *f. 1–51.* Chicago: General Biological Supply House. 1946.

Realizing that many people nowadays take the microscope for granted and do not really understand its operation, this booklet was compiled to help "teach the practical application of microscope theory, improve the young microscopist's technique and at the same time increase his range of operations." In seven short chapters, profusely illustrated, the author describes simple experiments, requiring little apparatus, which will give one a better appreciation of the possibilities of his microscope. First he illustrates results which may be obtained with a drop of glycerine as a lens. Then he shows graphically the difference between magnification and resolution, the meaning and importance of numerical aperture as applied to a compound microscope, and the necessity for proper control of the beam of light. Special attention is given to the correction of spherical aberration and to the proper illumination with each type of objective. A chapter on the use of light filters and polarized light brings out some interesting contrasts and shows how the use of these aids may improve the image or reveal new details. Brief discussions of stereoscopic vision with a monobjective binocular microscope, three-dimensional photomicrography, and micro-projection suggest interesting lines to follow for more elaborate uses of the microscope. A number of references are cited for more extended study of the topics discussed. In providing a brief, easily understood introduction to the principles underlying the microscope and its proper use, the author has very admirably accomplished the purpose he set for himself.—HAROLD H. CLUM.

INDEX TO AMERICAN BOTANICAL LITERATURE

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(exclusive of fungi)

(See also under Genetics: **Castle; McFadden & Sears; Sherman**; under Ecology: **Ciferri**)

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